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Science and public pleasure

In a recent interview on BBC television, Dr J. Bronowski was talking about job satisfaction. "Scientists and prostitutes", he said, "have one thing in common—they both get paid for doing something they enjoy." Many would question the enjoyment that Bronowski attributes to prostitutes but surely scientists enjoy themselves.

We looked, a few months ago, at the fun side of enjoyment—the scientific jokes of various guises—and this need not detain us here. What we now consider is the more lasting aspect of enjoyment; job satisfaction, but more than that. Some jobs offer satisfaction to the doers but hardly to others, certainly not to the general public. One does not, for instance, expect a stockbroker, however personally satisfied by his job, to look for any sort of public acclaim. At the other end of the spectrum are those who offer some sort of pleasure to the general public, not necessarily to their own satisfaction (for example, prostitutes, troubled poets and so on). Where do scientists, and in particular research scientists, fit into this scheme? There is clearly not a preordained slot in a satisfaction diagram for scientists but the question is valid, partly because recruitment into science depends substantially on how much practitioners are perceived to enjoy themselves and partly because the question of a more public delight in one's work is something about which scientists are ambiguous.

There are, doubtless, many surveys carried out among scientists of how happy they are with their job. Such surveys are of little use if they comprise no more than a question, 'Are you happy in your work?' Few will admit to being unhappy; not only is it an admission in itself but it also reveals the vulnerability of the highly trained person who has few options to move other than within his specialisation. The question 'do you give other people pleasure through your work?' is a more difficult and telling question. It is also one which will be dismissed by many as irrelevant. 'Science is not show business.' But it is, and there is nothing disreputable about show business, which comprises music as well as music hall, theatre as well as circus.

Many myths about scientists have been exploded in the past ten years and among these has been that the scientist has some sort of detachment both with regard to his personality and to his way of doing things. *The Double Helix* must surely have been the most eloquent source of this demythologising. And yet the emotions which Watson so vividly and honestly portrays are not confined to those who make the greatest of discoveries; in a sense they are even stronger among those whose names have not become household words because the final accolade is still to come (or never will). It is inconceivable that a vast majority of research scientists do not live with thoughts and hopes of wide recognition and acclaim, and positively revel in such exposure as comes their way. Which is what show business is about.

The acclaim is, of course, somewhat rarefied by the standards of the world. Some may be perfectly happy with the occasional paper in a learned journal, but they are a minority. A paper in *Nature*, a review article in *Scientific American*, these are more attractive as an opportunity to display one's skills to a wider public, even though the wider the public the less discriminating they can be. And the ultimate for most is publicity before a universal and thoroughly (indiscriminating audience) through the mass media.

It is at this level that most scientists become studies in ambiguity. Pleasing as it is to see oneself in print or on television is it not a trifle vulgar, and should the media not be kept at a discreet distance? Surely not. The general public can certainly take a delight in things it does not understand. There are many levels on which one can enjoy a performance of *Hamlet* and there are similarly many levels at which a scientific accomplishment can be savoured. Further, the scientist who refuses to have anything to do with the media is letting in the professional commentator, already at one remove from the field. Misrepresentation is then not the commentator's fault but the scientist's.

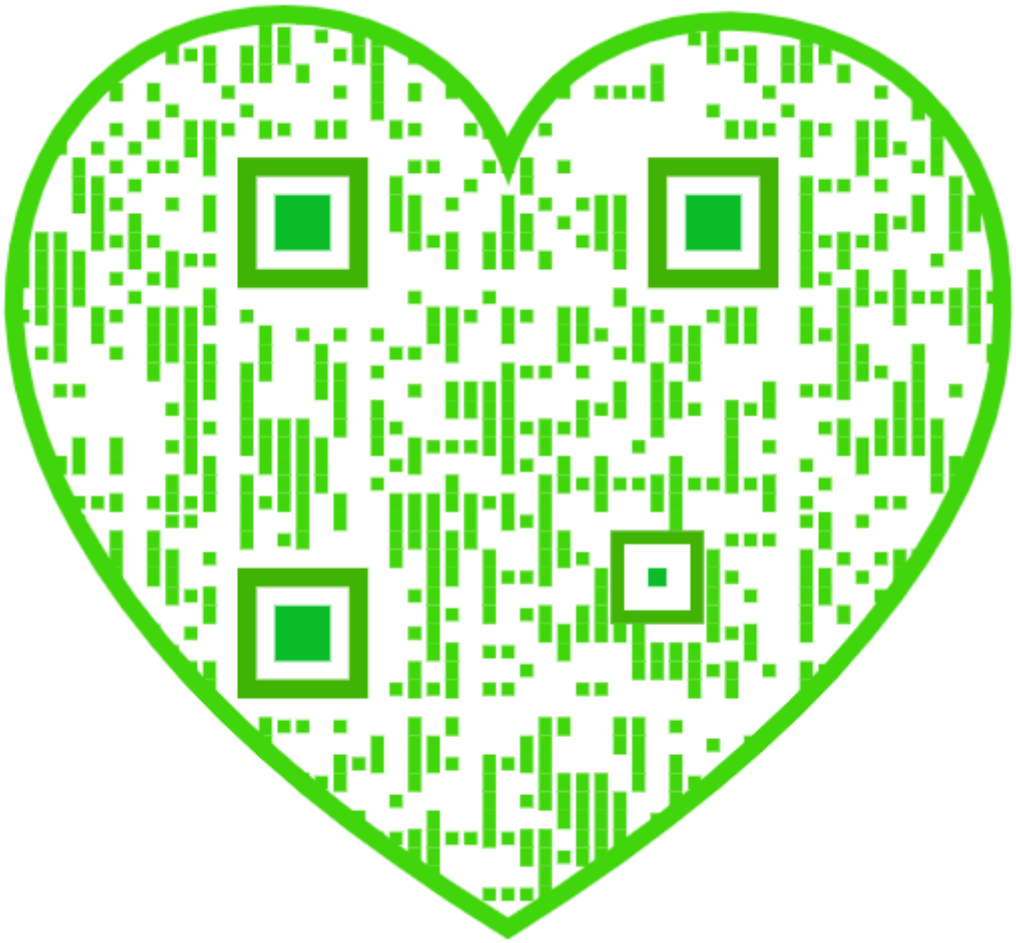
Ability to reach a more general public is not at present uniformly spread over the sciences. Although some fields seem to command wide and appreciative audiences, others never seem to project themselves. Chemistry is a case in point. Many chemists entertain serious doubts that their subject can be of interest (other than financial) beyond their own community. This may be true, but it is worth regular efforts to find out whether chemitsry has an appeal.

100 years ago



A Reuter's telegram from Aden, of March 23, states that the steamer *Calcutta* arrived there from Zanzibar on the previous day with the body of the late Dr. Livingstone. We fear this must be regarded as final, and as shutting out any further hope; we can only now do all possible honour to those remains which the doctor's faithful servants have so religiously preserved. A letter recently received from Zanzibar, by Mr. R. A. Laing, states that the body, after having been exposed to the sun for a month to dry, and then packed in a hollowed tree, was wrapped round with cloth, and the natives carrying it supposed it a bale of cloth, or kaniki.

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Centre for the practice of theory

Next September, the International Centre for Theoretical Physics in Trieste will celebrate its tenth anniversary. In a decade it has become a major, but still unique, feature of the scientific world. Professor John Ziman, of the University of Bristol, describes its problems and successes.

THE International Centre for Theoretical Physics (ICTP) was inspired by Abdus Salam, professor of theoretical physics at Imperial College, London, who recognised, from personal experience, the need for a place where young and promising research workers from developing countries might come from time to time to "recharge their intellectual batteries". Salam persuaded the International Atomic Energy Agency (IAEA) to create such a centre, which came to Trieste at the invitation of the Italian government and of the City of Trieste.

After some years in temporary premises in the centre of the city, the ICTP moved in 1968 to an elegant building paid for by the regional government and the city. This building overlooks the Adriatic, about 8 km north of Trieste along the main coast road, and has offices and lecture rooms for about 100 physicists at a time. In the summer, naturally enough, the place is crowded, but the building is now in fairly constant use throughout the year. Unfortunately, domestic accommodation has to be found in hotels or lodgings in the city, which means a lot of bus travel; there are long term plans to provide a hostel nearby. The centre has its own excellent library, but the nearest computer facilities are in the University of Trieste. The material facilities are better designed for high-minded individual cerebration than for team research on dirty facts.

Noble laureates and brash young physicists

About 5,000 physicists from 90 different countries have visited the ICTP since it was founded. Of these, at least 1,000 have worked there for periods of several months or more. Visitors range from academicians and Nobel laureates, on courtesy calls of a few days or weeks, to brash young theoretical physicists from developing countries on nine-month fellowships or associateships. But the centre is not an 'institute' with a permanent academic staff committed to a long term research and teaching programme. By arrangement with Imperial College, Professor Salam, the director, spends two-thirds of his time at Trieste, and Paolo Budini, the deputy director, is also a professor at the University of Trieste. Other Trieste professors work regularly at the centre, but do not dominate its activities. In principle the scientific programme of the ICTP is guided by a Scientific Council, nominated by the various sponsoring agencies, which meets for a couple of days annually; in practice, the initiative rests almost entirely with Salam and Budini, in cooperation with various senior scientists who have become involved in the programme of the centre over the years. A small, hard working technical staff provides administrative, secretarial, library and printing services.

The precise constitution of the ICTP as an international organisation is not easy to explain. Legally it is a part of the IAEA and UNESCO, although the administration is mainly done by the IAEA. Financial support is shared in about equal proportions by the IAEA, UNESCO and the Italian government. Some matters have to be referred to the headquarters of the IAEA at Vienna and to UNESCO in Paris: others are dealt with by a 'consortium' of local Italian academics and officials. Funds for major activities at the centre also come from the United Nations Development Programme (UNDP), the Swedish International Development Agency (SIDA) and, until recently, from the Ford Foundation. Various overt or hidden subsidies come from such bodies as national scientific agencies. An income of about $1 million a year looks healthy but policy changes, earmarking, exchange fluctuations and other sources of variance or constraint make each year's budget a work of art. The best that can be said of this confused situation is that the centre itself is not burdened with detailed external controls or a heavy bureaucracy, and has been free to find an ecological niche for itself in the jungle of international organisations—who themselves have shown wisdom and restraint in not crushing this strange little beast underfoot.

Changes from experience

Originally, the main purpose of the centre was to provide shelter and intellectual companionship for a number of 'associates'—theoretical physicists of proven competence, taking temporary leave from their academic or government jobs in developing countries for a few months each year to undertake advanced research and to refresh their minds by contact with their peers. This research was to be directed and inspired by distinguished visiting scientists from more advanced nations, for example American professors on sabbatical leave. Naturally enough, opportunities for advanced seminars, small conferences and short study courses would also be exploited as the occasion arose.

It must be admitted that this initial plan has been overshadowed and modified with experience. The quality of the research produced by associates at the centre was very uneven and their need for very close personal supervision could not always be met, simply because there were not enough senior people who could come to Trieste for long periods without financial support. Many of those chosen as associates were ill prepared for self-winding research in an atmosphere of scholarly independence. This defect is difficult to avoid when candidates must be selected on the basis of written applications from a distance and is particularly serious with applicants from small countries with very weak scientific traditions. In some fields of physics, therefore, the associate scheme is now being used mainly to help research workers whose ability has been confirmed by the part they have already taken in other ICTP activities.

Contribution

The research output of the centre (about 100 papers a year, of which half are by physicists from developing countries) is, in fact, quite substantial. In certain specialised fields of elementary particle theory, such as group theoretical methods, the use of non-compact groups and the connections with the theory of gravitation, it is the leading place in the world. In other fields the preprints circulated from the centre often represent important preliminary stages in investigations that are continued and completed elsewhere.

But the contribution of the ICTP to physics should not

be judged from the research actually done there. In recent years, much more emphasis has been given to 'extended seminars', 'winter colleges' and so on, where whole fields of advanced physics are systematically expounded by invited lecturers of the highest international reputation. For each such course, scholarships are given for 30–50 participants from developing countries to come to Trieste for 2–3 months. These courses are open, without fee, to appropriately qualified scientists from all countries and are well patronised by physicists from West Europe. Trieste is geographically well placed to receive physicists from East European countries, whose participation in the ICTP would be even greater if it were not severely limited by exchange controls and other administrative restrictions on travel. The Soviet contribution to the scientific programme has not been as great as was originally hoped but there have been long periods of collaboration at the centre between teams of plasma physicists from the Soviet Union and the United States.



A group of physicists at the ICTP, Trieste.

Many of the best physicists in the world, senior and junior, may be found talking together in the seminars, 'congressini', and 'research workshops' that arise spontaneously during or after each extended course. The centre has thus become a major forum, or market place for the international physics community. Personal contacts are established, ideas are exchanged and new developments communicated in a more leisurely and relaxed environment than the conventional scientific conference or summer school. The general atmosphere is serious and business-like; discussion ranges from the technical problems of mathematical physics to the role of the scientist in a developing country. The typical participant is about 30, has his PhD and several published papers to his credit, and is already carrying responsibility as a university teacher or in a newly formed research group in his own country. But the name on the office door speaks of Nigeria or Brazil, of Pakistan or Korea, rather than the United States, Europe, the Soviet Union or Japan. This is one of the few international scientific institutions in the world that is not dominated by people from economically advanced nations—and that is not just a political talking shop.

In founding the centre, Salam naturally concentrated on his own field of research, the theory of elementary particles. This remains a major component in a scientific programme which now includes regular activities on the physics of nuclei, atoms, molecules, plasmas, condensed matter, living organisms and the Universe, and on mathematical topics such as computational methods, global analysis, and fluid dynamics.

Highbrow theoretical meetings such as an International Symposium on Contemporary Physics are balanced by courses that get down to hard experimental facts, such as the 1974 Winter College on 'Surface Science'. The centre now caters for the experimental physicist who wants a simple explanation of the theoretical background of his research as well as for the 'theoretician' with his abstract mathematical models. The proportions of the mix are mainly determined by the relative enthusiasms of various individuals who are willing to organise various activities, but would not perhaps be very different if there were a long term plan, based on firm financial guarantees, thought out carefully by a representative committee.

The facilities and programme of the ICTP are now much valued by the world physics community. It is well known as a good place to work at for short periods and the high standard of the extended seminars is coming to be appreciated both by potential lecturers and by their audience. But it still suffers from a shortage of active personal support and practical commitment over long periods from those leading physicists who could help so much in the scientific direction of its activities. Labour is an essential component of the fine ideal of international scientific collaboration.

Serving interests

Does this programme serve the genuine interests of the developing countries? The argument that the real need is for engineers, doctors, technicians and other practical experts is valid on the whole, but ignores the contribution that quite small numbers of well informed and competent academic research workers can make to the solution of technical problems, to educational standards and to cultural life.

Over the years, the activities of the centre have slowly shifted towards the more applicable branches of physics, in response to the political demand for greater 'relevance' to social needs. Indeed, this shift has not been altogether passive: physicists from developing countries meeting together in Trieste as a distinctive group begin to realise for themselves that the pursuit of pure science should not be regarded as an end to itself. In the extended seminar programmes, the intellectual continuity is emphasised between, say, the theory of elementary excitations in covalent solids and the technology of semiconductor devices. This provides a bridge over which the young scientist may move from the academicism of the old-fashioned physics curriculum to the more practical territories of engineering and industry.

A natural extension of the programme of the centre is to support similar activities in other regions of the world. This can only be done on a very limited scale, without permanent facilities, but should eventually strengthen the social and intellectual links established between physicists of various countries when they meet at Trieste. The ICTP cannot, of course, replace the great centres of physics research that exist in advanced countries and that are growing up in many developing countries, and can only supplement the specialist conferences, travel scholarships, regional summer schools and so on that already bind together the world scientific community. But because it is a place where the scientist from a developing country feels he comes as of right, and where people work hard together, on an equal footing, to the highest intellectual standards, it makes a unique contribution to international cooperation and national development. It thus represents a sound prototype for similar institutions in other fields of science and scholarship.

international news

The milk of human unkindness

John Hall



WAR on Want, a British agency pledged to make an urgent political issue of poverty, has accused western manufacturers of causing disease and malnutrition by promoting sales of powdered milks in Third World countries where their use is both unnecessary and unsuitable. In a report called 'The Baby Killer' War on Want says that babies in developing countries are dying because of bottle feeding, and many that do not die are drawn into "a vicious cycle of malnutrition and disease that will leave them physically and intellectually stunted for life".

The basis of the accusation is that sophisticated western marketing methods are being used to persuade mothers in Africa, Asia and Latin America to abandon breast feeding in favour of artificial substitutes. While these substitutes might make perfectly adequate infant foods under proper hygienic conditions, and play an important role in disaster situations when there is no alternative, they become dangerous when used continuously by mothers incapable of reading instructions and in kitchens where there is no clean water for mixing and sterilising.

According to the report the baby food industry stands accused of promoting its products in countries which can't use them properly, and of using advertising and supplying samples and free gift gimmicks that persuade mothers to give up breast feeding, often with fatal results for their children. The mothers often receive the impression that information they receive from commercially based advisers is something in the nature of nutrition education, and 'The Baby Killer' singles out as particularly unethical the use of medically unqualified sales girls, dressed in nurses' uniforms and offering a sales pitch dressed up to look like objective feeding advice.

Other practices which the report describes as unethical and immoral include the encouragement given to mothers to bottle feed while they are still breast feeding satisfactorily, and before there is any need for supplements and the payment of qualified nurses on a sales-related basis belying their educational role. According to a source within the industry, these nurses are not only paid a commission on sales, but are given an additional incentive in the form of a promise that they will be fired if they don't meet their targets.

The problem of bottle-carried infection is well known in the Third World, if not widely publicised in the west. Even in Britain, where standards of living are comparably high, and most families live in hygienic conditions, hospital statistics show that babies still suffer from infections passed on from feeding bottles. War on Want points out that in the slums of Latin America and the squatter suburbs of Africa, where comparable conditions are hopelessly beyond reach, the choice of an artificial substitute for breast milk is in reality a choice between health and disease—disease caused by an infection or by long term malnutrition.

Breast milk cannot be improved upon as far as infant feeding in underdeveloped countries is concerned: it comes ready mixed, needs no warming or sterilising, leaves no dirty pots and bottles to be washed, and is almost always on tap from its carefully evolved, unbreakable containers. What's more, it contains an appropriate mix of proteins and fats, and seems to offer young infants protection against disease. In Chile, where 20 years ago 95% of one-year-olds were breast fed, the figure is now down to 20% at two months old: at the same time researchers have found that Chilean babies who were bottle fed during the first three months of their life suffered a mortality rate treble that of breast fed infants.

The Protein Advisory Group of the United Nations (PAG) has produced a table expressing as a percentage of the national minimum wage the cost of artificially feeding a baby. It shows that in Nigeria the cost of feeding a three-month-old infant is about 30% of the minimum urban wage, and by the time the child is six months old the cost will have risen to a crippling 47%. In Pakistan, comparable figures are 40% of the wage at three months, 62% at six months. Not surprisingly, mothers attempting to bottle feed their children at this rate of exchange try to eke out their supplies by overdilution, so that the children to whom the over-watered milk is fed receive an inadequate intake of nourishment. They are left weak, vulnerable to infection, and often end their lives falling prey to wasting diseases like kwashiorkor.

The PAG manual on feeding infants and young children recognises the problem when dealing with powdered milk supplies. "In the less technically developed areas of the world", it says, "immediate and serious basic difficulties attend attempts to artificially feed young infants on a cows' milk formula. These include lack of sufficient money to buy adequate quantities, poor home hygiene (including water supply, fuel, feeding utensils, storage etc) and inadequate nutritional knowledge of the mother. Under these conditions, usual for the

majority in less developed countries, artificial feeds mean the use of too diluted, highly contaminated solutions of cows' milk, resulting at the best in undernutrition, at worst in marasmus and diarrhoeal disease".

And Dr James Farquhar, a reader at the University of Edinburgh and consultant paediatrician at the Royal Infirmary of Edinburgh, reinforces the point in terms which virtually precis War on Want's message: "It is clear to all but those who will not see", says Dr Farquhar, "that informed, adequate and relatively safe bottle feeding must follow, or at least accompany but never precede, literacy, education, infection-free water supplies, sanitation and a standard of living which permits the purchase of enough baby foods, equipment and the means of sterilisation".

The priorities for dealing with the trend away from breast feeding, described by the report as a trend which can only have calamitous consequences, have been stated by the PAG in the following terms:

"The major overall need is to alert governments, health services, nutritionists and the food industry to the emergency situation likely to develop in urban areas in the near future. Its implications are not only the certainty of rising mortalities from almost epidemic marasmus and diarrhoea but also the economic burden of curative services and of obtaining breast milk substitutes on a large scale as well as the long term consequences of the effects of recovered cases of infantile malnutrition on the intellectual level of the community".

War on Want says the aim of its 'Baby Killer' report is to achieve a solution to the problem by opening up the subject to public debate, and it makes recommendations both to the industries and the governments involved. The companies producing dried milk should follow the Swedish example, they say, and refrain from all consumer promotion of breast milk substitutes in high risk communities. They should cooperate constructively with the international organisations working on the problems of infant and child nutrition in the developing countries, and they should abandon promotions to the medical profession which may perform the miseducational function of suggesting that particular brands of milk can overcome the problems of misuse.

Governments of developing countries are recommended to take note of the PAG's advice on national nutrition strategies: and they are advised, where social and economic conditions are such that proprietary infant foods can make little useful contribution, that serious consideration should be given to ending their importation, distribution, and/or promotion.

Astronomers meet administrators

Edward Phillips

THE discussion meeting on astronomy policy arranged by the Royal Astronomical Society in conjunction with the Science Research Council (SRC) turned out to be a staider affair than might have been anticipated, probably because the most vociferous critics of British astronomy are working outside the United Kingdom. There were no outbursts *à la* Burbidge or Evans. Nor were Sir Fred Hoyle, who two years ago seemed to have fallen foul of British astronomy politics, or Professor Ray Lyttléton, who has been a critic of astronomy policies, at the meeting.

And with the meeting being held in the afternoon of Friday March 1, when the final election results were still coming in, the gathering was mindful of pre-election prophecies that a no-result election would be bad for the economy and therefore bad for big science. The main SRC representatives, Sir Eric Eastwood (Chairman of the Astronomy, Space and Radio Division) and Dr H. Atkinson (Head of the Astronomy, Space and Radio Division) made furtive glances at a blackboard showing the latest election figures whenever anyone mentioned expensive projects.

The election notwithstanding, Dr Atkinson was hopeful that the budget for the Astronomy, Space and Radio Board during the coming financial year will be about £20 million (something less than the cost of making one Concorde), divided up as follows: £4.7 million as the subscription to the European Space Research Organization) (ESRO), £2.2 million for minor grants, £2.8 million for SRC establishments such as the Appleton Laboratory and £10.3 million for major facilities such as the projected Mark VA radio telescope for Jodrell Bank, the Northern Hemisphere Observatory (still no site fixed), an infrared flux collector, the Isaac Newton and Anglo-Australian telescopes, and various satellites, rockets and balloons.

The main news to report was that the two crucial committees of the board, the Astronomy Policy and Grants Committee and the Space Policy and Grants Committee, are soon to be folded up. Instead of these two committees which divide the board's parish on the basis of the technique used—astronomy that makes use of satellites would come under the space committee, for example—the SRC is to establish three committees whose interests are defined in scientific terms. An Astronomy I committee will concern itself with radio, X-ray and γ-ray astronomy and with cosmic rays, high energy astronomy in other words. Astronomy II will deal with low energy astronomy, including optical, infrared and ultraviolet astronomy. A Solar System committee will be interested in the Sun, Moon and planets, in solar-terrestrial relations, the Earth's ionosphere and so on. Astronomers will be waiting to see whether new arrangements prevent the perennial research council disease of grant applications falling between two committees.

The meeting also heard that some decision on the much-discussed Northern Hemisphere Observatory is expected this autumn. Professor D. W. N. Stibbs, Chairman of the Astronomy Policy and Grants Committee, said that the SRC is thinking in terms of having three telescopes at the observatory, with apertures of 1½, 2½ and 4½ metres, the largest one possibly having an alta-zimuth mounting. With Tenerife now

Musical chairs again?

JUDGING from recent advertisements, the British astronomical community is once more to be thrown into turmoil. Four posts are available at the University of Sussex from October 1974, an unspecified number of "positions" at the Institute of Astronomy in Cambridge from the same date, and Professor H. Brück's retirement will, a year later, result in the appointment of a new Astronomer Royal for Scotland and Regius Professor of Astronomy in the University of Edinburgh. No doubt astronomical administrators must also have it in mind that the caretaker Director of the Royal Greenwich Observatory (RGO) also reaches retiring age in the not too distant future.

In recent years, there has been a remarkable shuffling of astronomers around Britain, in some cases back and forth between the same universities; a possible conclusion to be drawn from these musical chairs is that there is a lack of talent at the highest levels. It is certainly difficult to think of two people both acceptable to the establishment and willing and able to handle the jobs at the RGO and Edinburgh. It might be worth reflecting on the possibility that the insecurity engendered by the appointment of researchers for "one, two or three years", and the resulting shuffling sideways at frequent intervals, could be one contributory factor in discouraging the development of sufficient talent both able to do the top jobs and willing to stay in Britain.

apparently out of the running for political reasons, the choice of site seems to be settling down to either Hawaii or Madeira, the Atlantic Island belonging to Portugal. Professor Merle Walker of Lick Observatory, California is acting as a cosultant for the SRC during the selection process.

The decision between the two sites seems to be finely balanced. Seeing conditions are generally accepted as better on Hawaii, but by how much better is uncertain, and with the cost of an observatory on Hawaii being higher than Madeira it may yet turn out that, photon for photon, Maderia gives better value.

In the past the Science Research Council has had a reputation for keeping its ideas to itself until much too late in the day. The holding of this special meeting was therefore held to be a step in the right direction, even though it was noted that in the case of the reorganisation of the committee structure the decision had already been taken. Some participants, notably Professor Tom Kaiser (University of Sheffield) wanted even more disclosure of the way the SRC goes about its business and Professor Martin Rees suggested that there be a series of smaller follow-up meetings to discuss particular aspects of the SRC programme.

Water storage in the Wash

Eleanor Lawrence

SINCE 1965 the Water Resources Board has been investigating the possibility of freshwater storage in the Wash estuary on the east coast of England. The board has recently published the interim report on the feasibility study for a proposed scheme costing £140 million, which, if implemented, will eventually provide a major new source of water for the heavily populated south-east corner of England.

A desk study completed in 1970 decided that at present a full barrage enclosing the Wash was technically impossible but authorised a practical study of the first stage of a scheme in which freshwater from the rivers Great Ouse, Nene, Welland and Witham, which flow into the Wash, would be pumped into a system of three or four artificial reservoirs built just offshore. This feasibility study, which includes an ecological survey and the construction of trial embankments, is expected to cost £2.6. million over about six years.

The area has now been completely mapped by aerial, hydrographic and land surveys. And three sets of models are under construction at the Hydraulics Research Station at Wallingford to study conditions likely to arise from building structures in the Wash and taking water from the inflowing rivers. Geological and geophysical surveys are now complete and have not revealed any major problems.

A trial inshore bank (the bank bounding the landward side of the reservoir) was successfully completed in 1972 and showed that it would be possible to construct the banks using hydraulic fill piped from the existing foreshore sediments. This bank was built entirely from the land and it was intended to construct a trial offshore bank during 1973. In the event no suitable tender was received and so construction was postponed. In view of the present financial position, however, this proposal is being completely re-examined by the government and may be cancelled, especially in view of the board's recommendation that the Wash scheme need not be considered for development until the late 1990s.

Apart from this, the other major outstanding item is the programme of hydraulic model tests. This should be completed by 1975, says the board, and it should then be possible to pronounce on the feasibility of the first stage of the scheme, a reservoir covering 1,700 acres near the mouth of the Great Ouse.

Ecological surveys coordinated by the Natural Environment Research Council have been continuing since 1971. The Wash covers 232 square miles and contains some of the finest salt marsh and mud flat habitat in Britain. The Ouse Washes which are most likely to be affected by the development have been designated by the Nature Conservancy Council as of grade I scientific interest and are included in a register prepared by the International Union for the Conservation of Nature as being of outstanding significance.

The Wash itself is one of the most important breeding and feeding grounds in Britain of the Common Seal which, despite its name, is not so common now. As their main feeding grounds are in the outer Wash, seals are not likely to be affected by development, at least in its initial stages, unless salinity changes affect the type and abundance of food species.

The surrounding mud flats are a particularly important feeding ground for overwintering waders and wildfowl. Monthly counts suggest that the number of waders centred on the area can reach 300,000. One of the four proposed sites for the first reservoir, Bulldog Sands to the east of the mouth of the Great Ouse, is one of the largest feeding grounds for the wading birds whereas another, on the west of the river mouth would be less acceptable to the shellfish industry, which produces about £250,000 worth of various shellfish from the Wash each year.

Final conclusions on the feasibility and siting of the reservoirs must await the results of the hydraulic model tests, final costings and the possible construction of the offshore bank. The report promises that ecological considerations will be taken into account and the Nature Conservancy Council plans to publish a separate report on overall conservation when the final results of the engineering studies are known.

Physics for physicists

John Hall



PROFESSOR Frederick Guthrie founded the Physical Society in 1874 "for showing new physical facts and new means for showing old ones, for making known new home and foreign discoveries", and one of his regular teaching aids was a compartmentalised box containing specimens of the actual insects, minerals or materials which were the subjects of his lessons. The point was that any number of descriptive talks on, say, cotton spinning, fell short of the mark if a pupil simply had no clue about the appearance, the textures, indeed the nature of raw cotton. These object boxes (origin of the term 'an object lesson') are among the early exhibits in a display of physics memorabilia forming the exhibition "Phonographs to Holographs" at the Science Museum, South Kensington, in honour of the Physical Society's centenary. (Incidentally, one of the historic object boxes pictured contains, under the heading "butterflies", a creature which bears a remarkable resemblance to a Cinnibar moth; but the spirit of the thing was clearly progressive.)

The exhibition runs to an engaging range of Victorian laboratory machines, resplendent in brass and mahogany, and

throws on a number of distinguished investigators sidelights which never made the pages of a text book; Sir William Thomson, for example, is characterised not only as a designer of precision electrical instruments but also as the inventor of a patent water tap. What's more, a good many of the old masters' experiments are reproduced as working models, in the museum's push-button tradition, though the precise value of the demonstrated principle is not always made clear. Kelvin's water dropper, for example, is a pleasant enough looking contraption which intermittently interrupts two streams of water, sends out a spray of droplets and emits a blue spark. An attached rubric explains that this electrostatic induction machine had no practical application, although it led to the design of the Replenisher and the Mouse Mill Generator. Which is all very well if you are acquainted with mouse mill generation, and know its significance (or otherwise).

In fact, as soon as the Victoriana has been digested, the exhibition is plainly a display for the specialist, and calculated to suffuse the museum's staple schoolboy customer with indifference. The explanation of the Josephson effect might well have been written in a foreign language and when a layman looks at a superconducting solenoid it does not really help to know that it has a field of 5.4 tesla with a current of 2,300 A. It does not even help to be told that you are looking at a superconducting solenoid. Similarly, two historic pieces of apparatus from the Cavendish Laboratory fall on stony ground if you have not the first idea of what bombardment means, or why it is nice to see the paths of atoms traced in a cloud chamber. C. T. R. Wilson's cloud chamber, described by Rutherford as "the most original and wonderful instrument in scientific history" does not begin to look as though it deserves such a testimonial, and neither does the written explanation supply a justification. A group of youngsters perusing the show on opening day gave about five seconds each to telephones through which one could hear the voices of Wilson and Rutherford describing their work. Five seconds was really not long enough to catch their drift but it was just about long enough to establish that you did not really understand what they were talking about.

So, if you are a physicist, this is an exhibition which you will discover to be packed with nostalgic gadgetry (*pace* Wilson and his cloud chamber) and if you are not a physicist it is an exhibition which tells a tale of the sound and the fury (disintegrating the nucleus of an atom surely qualifies for that description), but may not signify too much. The title of the show springs from the fact that during the first decade of its existence the most striking foreign discovery that the Physical Society saw was Edison's phonograph; a highlight in recent times was a demonstration of Gabor's Nobel prize-winning holograph. And there at the entrance to the exhibition sits a holograph of Gabor himself, looking for all the world like a Tussauds version of the multiple murderer Reginald Christie. Two more exhibits which deserve mention as eye-catching and reasonably self-explanatory spectacles are a scanning electron misroscope (the Cambridge Stereoscan 600, whose blown-up pictures would have looked all that much better in colour) and a pair of motorised mirrors which track back and forth on rails in an experiment measuring the velocity of light. On the whole, this exhibition supplements Rutherford's definition of all science being either physics or stamp collecting. It is also semantics.

First Australian-Soviet science agreement

Peter Pockley, Sydney

THE first agreement between Australia and the Soviet Union for cooperation on science and technology was initialled in Canberra at the end of February. At this stage the formal agreement has been expressed only in the most general terms.

A delegation of three Soviet science administrators paid a preliminary visit to Australia to discuss possible projects of cooperation. The Soviet delegation was at a high level—representing the State Committee, USSR Council of Ministers for Science and Technology were Mr L. N. Yefremov (First Deputy Chairman) and Mr V. A. Kuzin (Head of the Department of Foreign Relations); representing the USSR Academy of Sciences was Mr S. G. Korneev (Chief of the Directorate of Foreign Relations).

The agreement is seen as a quiet first, but significant, step in the Australian Labor government's diplomatic moves to equalise, as far as possible its formal relations between the western, eastern and Asian blocs. Australia has had a binational agreement with the United States for scientific and technical cooperation since 1968. Australian science also has strong links with Britain through the Anglo–Australian Telescope Board and a cooperative programme with Japan for its geostationary meteorological satellite is being evaluated. Soviet and Australian scientists will now work towards the signing of a detailed agreement in Moscow later this year. The Australian Department of Foreign Affairs has recently appointed its first Senior Adviser on Science, Technology and the Environment, Mr Guy Gresford, an Australian who was formerly Director of Science and Technology at the United Nations.

Linear motors and the EEC

John Wilson

EIGHT manufacturers of linear motors met recently in Brussels, at the request of the Commission of the European Communities, to work out joint proposals for the development of a high power motor suitable for a fast European inter-city transport system.

The companies, representing France, West Germany and the United Kingdom, outlined suggestions for future research but also indicated that the commission must identify the objectives of such a programme more precisely before close collaboration can begin. It is now up to the commission to assess the opinion of the various governments and transport authorities concerned and produce some formal proposals with which the manufacturers can come to grips.

The commission feels strongly that any inter-city transport system for the 1980s, which would involve travel at speeds >300 km h^{-1}, should be planned on a European basis. Whatever means are used to support the vehicle—wheels, jets of air or magnetic suspension—a high power linear motor is the obvious choice for the traction unit as it overcomes the mechanical difficulties of transmitting sufficient force to the track.

The 'club' of manufacturing companies, including Tracked Hovercraft, General Electric Traction and Linear Motors, first met in June last year and expressed their readiness to embark on a cooperative venture. According to the commission, a good deal of quiet consultation took place afterwards, and that formed the basis of the recent proposals. These are that three cross-company research teams should study the synchronous, asynchronous and transverse-flux motors, respectively; another should examine the problems of collecting electric current at high speed; and a fifth should investigate control systems for the motor.

At first there would be some parallel development of all the motors until a particular type or types were chosen for construction. Then these would be built and tested to evaluate the design.

But these proposals represent a very cautious stage in the negotiations between the members of the club because, although the commission tentatively estimates that it will spend about £2

million on the research programme over the next two years, no accurate costing has yet been attempted. The companies plan to contribute to the construction and other costs as the commerical stage of the project draws nearer but they hope that the commission will foot the entire bill until it is decided which type of motor will be developed.

There is also some concern that the various governments can reach agreement on the sort of system they want as readily as the commission seems to suppose. There is little point in the United Kingdom taking part, for example, unless the channel tunnel is in operation. Most population centres in Britain are so close together that, after the introduction of British Rail's new Advanced Passenger Train, journey times are unlikely to be significantly improved.

Unlike an existing rail system, where an individual company can take considerable initiative in its improvement, a new high speed network requires an enormous capital investment (in civil engineering costs alone) which places it firmly on a national, if not international, level. It seems surprising, therefore, that the commission has waited until now to begin consulting the potential users of the scheme. Without an agreed specification from the governments and others concerned, the technical collaboration of the companies, such as it is, will remain in a vacuum.

correspondence

Oops

SIR,—May we look forward to your tectonics correspondent's report on Aberdeen's 20 km move SSW[1]?

Yours faithfully,

JAMES R. GRIFFIN

Charles Griffin & Co Ltd,
42 Drury Lane,
London, WC2B 5RX, UK

[1] *Nature*, **247**, 416 (1974).

Population and politics

SIR,—The article "Is Population a Ministerial Responsibility?" is certainly a thought-provoking one, and makes the main point extremely clearly but it contains a statement which I think should not be allowed to stand unquestioned—that is "the current demographic position in Britain is that no serious economic or social problems which could be directly attributed to either its population size or growth rate are likely to emerge in the next 30 or 40 years". Further on Mr Cope points out that the Population Panel had argued the necessity of bringing home to the public the need to consider the aggregate consequences of their family building behaviour, in spite of the possible conflict of these consequences with individual preferences.

One point which immediately springs to mind is that, notwithstanding the very considerable publicity that has been given to the existence of family income supplements and similar benefits of this type, a large proportion of the group for whom they are meant are unaware of the existence of the benefits to which they are entitled, yet the limited evidence available in the report of the Population Panel suggests that this particular group, who form most of socio-economic group 5, are consistently among those with the highest reproduction rate. Although there is clearly scope for a great deal of work in fields relating the family's economic status to family size as matters stand (and with the example of one difficulty in communication of a real need being all too apparent) it seems to me highly premature—if not completely wrong—to suggest that continuation of trends such as this could not give rise to serious social problems over the next 30 or 40 years. An equal problem may arise by the over-reaction to publicity of groups such as socio-economic group 1.

Yours faithfully,

P. J. HEALD

Department of Biochemistry,
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Cigarettes and guilt

SIR,—Although any contribution to the problem of deterring cigarette smoking by pregnant women is welcome, the airing given to it in your leading article of February 15 seems to me to need correction.

Your suggestion that a different approach would have been more effective and humane is not one which the careful preparatory work which we did bears out. A substantial survey of the reaction of pregnant women to the approach adopted, and a smaller one concerning the use of the naked figure, established quite clearly that the message and its form were entirely acceptable to those for whom it is intended. Conversely, much experience of the use, mainly by other organisations, of soft and pretty material has established that it is, as regards topics as serious and difficult as this one, largely ineffective. Incidentally, the effort we are making represents not only a new dimension in a continuing campaign against smoking in general; it is a specific effort to tackle the most clearly avoidable of all the factors affecting perinatal mortality.

The "well informed doctor and nurse" to whom you refer are a vital element in the campaign. Almost all the general medical practitioners and midwives in practice, and a great many clinic staffs, have received a personal letter setting out the scientific case for the campaign and inviting participation in exactly the way you have in mind, using our leaflet if they so wish.

Finally, of course we agree with your suggestion that information should be given to school girls about this topic and, indeed, several others affecting their health and well being. But this is a different exercise to which we are addressing ourselves as a separate activity.

Yours faithfully,

A. C. L. MACKIE

The Health Education Council,
78 New Oxford Street,
London WC1A 1AH, UK

Survival of science

SIR,—And, in an editorial[1], grovelling low
Even at the base of Maddox' statua
Which all the while ran blood, great *Nature* fell,
O, what a fall was there, my countrymen!
Then I, and you, and all of us fell down,
Whilst bloody doomsay flourish'd over us.

Yours faithfully,

THOMAS H. JUKES

Berkeley, California

[1] *Nature*, **246**, 439 (1973).

news and views

Fermi surface of magnetic rare earths

THREE physicists at the University of Birmingham have reported direct measurements of the Fermi surface of gadolinium metal (Young, Jordan and Jones, *Phys. Rev. Lett.*, **31**, 1473; 1973). This is the first experimental observation of the Fermi surface of a magnetic rare earth element, although there have been some preliminary measurements reported by workers at the Ames Laboratory in the United States on a sample of non-magnetic lutetium (Hoekstra and Phillips, *Phys. Rev.*, **B4**, 4184; 1971).

In a metal some of the electrons are lost by the atoms to the conduction band. If these electrons were perfectly free they would have a quasicontinuous range of allowed energies up to a maximum energy, the Fermi energy. The electrons move through the periodic lattice of the positive ions, however, and these cause the formation of allowed and forbidden energy bands. In anisotropic three-dimensional materials the energy bands are also anisotropic and the Fermi surface, the energy surface formed by the Fermi energy in three-dimensional reciprocal space, also becomes non-spherical. These concepts of band structure and Fermi surface are vital in understanding the magnetic, electrical and thermal properties of metals.

There have been several very careful calculations of the band structure (Keeton and Loucks, *Phys. Rev.*, **168**, 672; 1968; Jackson, *Phys. Rev.*, **178**, 949; 1969; Dimmock and Freeman, *Phys. Rev. Lett.*, **13**, 750; 1964), and hence the Fermi surface, of the rare earth metals. In particular the magnetic properties have been interpreted in terms of very small variations of apparently insignificant parts of the theoretical Fermi surface (Evenson and Liu, *Phys. Rev.*, **178**, 783; 1969). Obviously before more detailed theoretical calculations are started there has been a need for some supporting experimental evidence.

Problems have arisen, however, because techniques which allow measurements of the Fermi surface require very pure single crystal materials. It is only in these samples that the electrons can traverse reasonable distances of the Fermi surface without suffering scattering. The rare earths have proved difficult to purify for a variety of reasons. The elements in the series all have identical outer electronic structure and hence chemical separation is difficult. Relatively pure samples of rare earth oxide can best be obtained by ion-exchange techniques and the metal produced from the oxide by a conversion to the fluoride followed by a calcium reduction of the fluoride. Added to this the individual elements are highly reactive, readily forming nitrides, hydrides, oxides and carbides both during preparation and on subsequent exposure to the atmosphere. Until very recently samples more than 99% pure with respect to all constituents were very difficult to manufacture.

The Birmingham workers have used the technique of solid state electrolysis in ultra-high vacuum (UHV) to improve the purity of their gadolinium samples, particularly with respect to the gaseous and carbon impurities. The initial material is taken in the form of a polycrystalline rod and placed between electrical contacts in a UHV system. A current of 500 A cm^{-2} is passed through the sample heating it to 1,100° C. The metallurgy of this process is not well understood but it is thought that during the electrolysis the oxygen impurities among others migrate to the anode end of the sample, while the hydrogen is simply released in the UHV heating. Hence a section cut from the centre of the sample is found to be purer than the starting material. If this subsample is reprocessed the purity improves still further. An added advantage of this purification process is that preferential grain growth takes place in the centre of the rod, producing single crystals with dimensions of some millimetres.

A simple, though for the rare earths a sometimes unreliable, method of checking the purity of the materials is to measure the resistance ratio (the ratio of the electrical resistance at 300 K to that at 4.2 K). The higher this value the purer the sample. The resistance ratio for these latest Gd samples (240) is an order of magnitude better than for previous material. The Ames lutetium had a resistance ratio of 21.

The de Haas–van Alphen technique, in which on cooling the sample to about 1 K it is found that the magnetisation is periodic with increasing internal magnetic field, has been used to study the Fermi surface of the pure Gd. The frequency of the periodicity can be associated with the frequency of a particular electron orbit around the Fermi surface. Young *et al.* were able to observe four separate de Haas–van Alphen frequencies for a magnetic field applied in the base plane of the hexagonal structure and a further three frequencies for a magnetic field applied along the hexagonal axis.

The higher experimentally observed frequencies can be associated with orbits round the theoretically predicted Fermi surface. For example, the theoretical Fermi surface is basically columnar in shape and oscillations around the trunk of the column can be observed and the diameter of the column estimated. The lower frequencies reported cannot, however, be explained with the theoretical Fermi surface at present established. As happens at regular intervals in developing areas of research, it seems that the tables have been turned. Until this paper was published theoreticians were demanding measurements of the Fermi surface to check their calculations. Now the experimentalists would like more sophisticated theory to explain their results.

Of course, these pure samples of single-crystal rare earth elements will start a new series of measurements on those physical properties that depend on purity. Already Wells, Lanchester and Lee at the University of Southampton have measured the specific heat of Gd at low temperatures and found no evidence for the anomaly at 3–4 K reported by previous workers. This anomaly can now be clearly attributed to Gd_2O_3 impurity present in the previous samples.

S. B. P.

Immunity to measles and canine distemper viruses

THE role of cell-mediated immunity in recovery from virus infection is a subject which is currently receiving considerable attention. It has long been recognised that dysgammaglobulinaemics—persons with deficiencies in their ability to produce humoral antibodies—recover normally from certain virus infections and can be immunised successfully against

smallpox. More recently, the key role of cell-mediated immunity in recovery from some of these diseases has been demonstrated experimentally by inoculating virus into mice whose ability to produce circulating antibody has been destroyed by the injection of cyclophosphamide. It now seems probable that, whereas recovery from enterovirus and arthropod-borne infections is due to the production of humoral antibody, recovery from herpesvirus, poxvirus and, possibly, myxovirus infections is largely a consequence of cell-mediated immunity, though cooperation between T and B lymphocytes has been demonstrated in several systems (see Allison, *Proc. R. Soc. Med.*, **66**, 1151–4; 1973).

The demonstration by Brown and McCarthy of a cell-mediated hypersensitivity reaction to canine distemper virus in dogs sensitised with measles virus (see page 344 of this issue of *Nature*) may throw new light on the nature of the immunity to distemper conferred by the inoculation of measles virus. Puppies born of distemper-immune bitches receive immunoglobulins in the colostrum and these not only protect against infection but also prevent successful immunisation with live distemper vaccine. Measles virus is not neutralised by distemper antibody and, provided sufficient is administered, can protect against subsequent challenge with distemper virus. Vaccination of dogs with measles virus may result in the production of some measles antibody but this has no neutralising effect on distemper virus *in vitro* and no distemper antibody is formed. This, together with the fact that protection is related to the dose of measles virus administered, suggests that the heterotypic virus functions in a similar way to the first dose of an inactivated vaccine, serving to stimulate a primary but not a secondary immune response (Prydie, *Vet. Rec.*, **83**, 554–9; 1968). On the other hand, a single dose of an inactivated vaccine does not usually produce a solidly-immune state and there is evidence that the efficacy of the vaccine is destroyed by treatment with formalin. Clearly, the mechanisms of heterotypic resistance cannot be satisfactorily explained on the basis of a humoral antibody response. The possibility that cell-mediated immunity may be the principal immune mechanism involved provides an exciting challenge for future study.

The work of Brown and McCarthy should also serve to stimulate further work on the mechanisms operative in recovery from canine distemper. There is considerable variation in the facility with which individual dogs recover from the disease and several attempts have been made to correlate recovery with the rate at which serum antibody is formed; it has been claimed that unless a neutralising antibody titre of about 1 in 100 (against 100–300 EID_{50} in the standard *in ovo* neutralisation test), is present by the fourteenth day after infection, the dog is likely to succumb. This view fails to take account of the evidence that dysgammaglobulinaemic children recover uneventfully from measles. Moreover, the neutralisation test measures only those antibody molecules which have not yet complexed with virus antigen; in an animal whose tissues are still actively producing virus, a low neutralising titre does not necessarily imply a deficiency in the humoral antibody response because a proportion of the antibody formed will be complexed with virus. Clearly, much remains to be done before the relative importance of different types of immunity in recovery from virus infection is fully understood. H.J.C.C.

Where do meteorites come from?

Nobody knows for certain where these large chunks of interplanetary matter come from. The ones that have been picked up from the Earth's surface have mineralogical and crystallographical characteristics which were probably produced during the early stages of planetary formation. These meteorites all seem to be transient members of near space, however, their life times as individual entities, away from their parent body, being several orders of magnitude shorter than the age of the Solar System.

Present theories fall into three main categories. First, meteorities could have an asteroidal origin, being produced directly as fragments of colliding asteroids in the asteroid belt or from those few asteroids which happen to cross the orbit of Mars. Knowledge of asteroidal bodies is very sketchy, but their orbits can be found easily and their diameters can be estimated from the intensity of the reflected light and from very careful direct optical measurements. Masses, densities and internal structure (apart from the measurement of the mass of Vesta by Hertz (*Science*, **160**, 299; 1968)) have all been estimated by assuming asteroids are made of meteoritic material; so one is back to where one started from. Two problems rear their heads with this theory. The short period of exposure to cosmic rays—even if erosion sputtering due to collision with microparticles in the Solar System dust cloud is taken into account—implies a demand on supply that is several orders of magnitude greater than the mass of debris produced by asteroidal collisions. It is also very difficult dynamically to bring a particle from the collision position in the asteroidal belt into the inner Solar System. The mean relative collision velocity of asteroids is around 5 km s^{-1} and an impulse of this order in the retrograde direction is required to change the orbit into one that intersects the Earth. The Mars asteroids are too few to yield sufficient debris.

Lunar ejecta has also been suggested as a source, but the mean geocentric velocity of this ejecta when captured by the Earth would be very low and also, because of pulverisation, large particles have little chance of survival. Öpik (*Adv. Astron. Astrophys.*, **4**, 301; 1966) calculates that about 0.2% of the meteorites have a lunar origin.

The third hypothesis has meteorites originating in comets. It must be remembered that as meteorites have such a short exposure age, their parent bodies must still exist and should be an observable class of celestial object. Stone and iron meteorites could come from cometary nuclei, the thick layers of icy conglomerate (in the Whipple model of the nucleus) shielding them from cosmic rays for most of their lifetime. The longer exposure ages of iron meteorites could be easily explained by the lower erosion rate of the surface of iron meteorites relative to stones. But there is little evidence of any correlation between meteor showers (which occur when the Earth goes through the toroid of dust debris produced by decaying comets) and the fall of meteorites. This could be due to the large gas pressure required to blow particles away from the gravitional attraction of the nucleus; in other words, large particles of the size of meteorites ($> 10^5$ g) might never get away whereas the smaller visual and radio meteors ($10^4 \rightarrow 10^{-5}$ g) do. Öpik finds that the biggest particles that can escape have diameters less than $17.3q^{-2}d^{-1}$ cm where q is the perihelion distance in astronomical units and d is the diameter of the nucleus of km. Comet Encke could therefore lose metre-sized chunks and large meteoritic fragments have been associated with the Taurid stream which originated from Encke. Of course the cometary origin still leaves the question of how the meteorites got into the nucleus in the first place.

One way of deciding between these theories is to analyse the structure of meteorites in more detail, hoping that additional knowledge might provide the vital clues. Results of this type of detailed analysis have been put forward in three recent papers in *Science*. MacDougall, Rajan and Price of the Department of Physics, Berkeley, University of California have found (**183**, 73; 1974) that the individual grains and chondrules in gas rich meteorites have high densities of particle tracks and severe track density gradients caused by low energy particle irradiation from nuclei accelerated by

solar flares. This irradiation damage was also non-uniform. They find that track density gradients exist not only on the particle surfaces but also at internal broken edges in the meteorite, indicating that the meteorite compacted more than 10,000 years after the grains were formed. The gradient is also so steep that only a very tenuous atmosphere ($\lesssim 10^{-3}$g cm^{-3}) could have intervened between the Sun and the meteorite. The non-uniformity of the irradiated features of the meteorites Kapoeta and Fayetteville are similar to those found in lunar soil. Grains also seem to have been shocked after formation, possibly during meteorite compaction.

Brownlee of the Department of Astronomy, University of Washington and Rajan have found (**182,** 1341; 1973) micrometeorite creaters, caused by hypervelocity impacts (>4 km s^{-1}), on the chondrule-like spherical objects in the meteorite Kapoeta. These craters are very similar to those found on lunar glasses. By comparing the meteorite with lunar materials, the authors conclude that the micrometeorite flux in the past was about an order of magnitude higher than it is at present, and that the chondrule spheres are droplets of once melted ejecta produced by cratering events on the parent body of Kapoeta. The rarity of these spheres in comparison with their relative abundance in lunar soils indicates that this parent body is of asteroidal size. Brownlee and Rajan conclude that the meteorite Kapoeta was formed by impact processes on an impact-generated regolith, this regolith being on an asteroidal-sized body; and that this process occurred during the latter stages of the accretion mechanism that formed the planetary bodies of the Solar System. It has also been found that the physical properties of interplanetary dust have not changed appreciably over the lifetime of the Solar System even though the flux was higher in the past. This implies a long term constancy of cometary phenomena in the inner Solar System.

Lange and Larimer (Center for Meteorite Studies, Arizona State University) have also been looking at chondrules (*Science,* **182,** 920; 1973), this time in the meteorite Ngawi. They conclude, however, that they were formed by flash heating and remelting of dust grains caused by high velocity (1–10 km s^{-1}) impacts in the cooling gas nebula that formed the Solar System.

It seems that chondrules could be formed right at the beginning of the Solar System or on the surface of an asteroid. Also the upper limit to the atmospheric gas pressure at the meteorite surface calculated by MacDougall, Rajan and Price does not exclude a cometary nucleus as the source of meteorites (do not forget that this nucleus is also being bombarded by interplanetary particles). So even now all the options are open and the origin of meteorites is still a mystery.

D.W.H.

Activation of latent leukaemia viruses

from a Correspondent

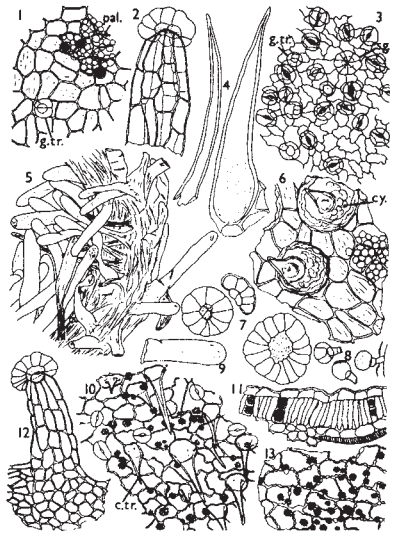
In 1970 Hirsch, Black, Tracy, Leibowitz and Schwartz (*Proc. natn. Acad. Sci., U.S.A.,* **67,** 1914) reported that graft-*versus*-host (G*v*H) reactions in BALB/c mice activated leukaemia viruses that otherwise remained latent or repressed. Hirsch and his colleagues then proceeded to demonstrate leukaemia virus activation during homograft reactions provided that immunosuppressive agents were also administered (*Science,* **180,** 500; 1973) and in mixed lymphocyte reactions *in vitro* (*Proc. natn. Acad. Sci., U.S.A.,* **69,** 1909; 1972). Since stimulation of lymphocyte proliferation by mitogens such as phytohaemagglutinin did not activate leukaemia viruses, these authors concluded that virus activation requires specific immunological stimulation.

Could the activated virus be responsible for the high frequency of lymphoreticular tumours found in mice undergoing chronic G*v*H reaction? Armstrong, Black and Richards (*Nature new Biol.,* **235,** 153; 1973) found that ultrafiltrates of spleens from mice undergoing G*v*H reactions induced leukaemia when inoculated into newborn mice. It is also well known that New Zealand Black (NZB) mice which suffer spontaneous autoimmune disease have a high level of endogenous leukaemia virus activity and a high incidence of lymphoreticular tumours.

It is becoming evident that the leukaemia viruses associated with chronic immune reactions in mice have a peculiar host range. They can neither be classified with the N-tropic nor B-tropic murine leukaemia viruses because mouse cells themselves are resistant to infection whereas certain cells of other species are susceptible. The virus of NZB mice has been studied by Levy and Pincus (*Science,* **170,** 326; 1970) who found that it is non-infectious for mouse cells but infects rat cells. Levy now reports (*Science,* **182,** 1151; 1973) that similar viruses can be isolated from normal spleens and kidneys of NIH-Swiss and C57BL mice. These tissues were co-cultivated with non-producer NRK cells transformed by Harvey strain murine sarcoma virus, whereupon a pseudotype of the sarcoma virus was produced. This pseudotype infected NRK cells and possessed similar biological and immunological properties as the pseudotype obtained with virus from NZB mice. Immunosuppressed NIH-Swiss mice tissues gave rise to the highest titre of pseudotype. Scherr, Lieber and Todaro in the first issue of *Cell* (**1,** 55; 1974) report that a virus activated in BALB/c cells *in vitro* during mixed splenocyte reactions does not infect mouse cells but replicates most efficiently in a rabbit cell line. This virus is probably closely related if not identical to the viruses isolated by Levy. Thus a virus can be vertically transmitted in one species but when activated may only be able to infect cells of other species. Levy calls such viruses "xenotropic".

Another example of xenotropism is the endogenous feline virus which when activated replicates in human cells. This virus was originally detected after inoculation of RD cells, derived from a human rhabdomyosarcoma (McAllister *et al., Nature,* **235,** 3; 1972), into foetal cats *in utero* (again, an immunologically-undeveloped host) and was at first regarded as a possible human virus (see *Nature,* **244,** 252; 1973). A similar experimental model was used by Todaro *et al.* (*Proc. natn. Acad. Sci., U.S.A.,* **70,** 859; 1973) to isolate a virus from NIH Swiss mice which had been immunologically suppressed with antithymocyte serum and inoculated with RD cells. This virus, AT-124, contains group-specific antigen and RNA-dependent DNA polymerase related to murine C-

Vegetable anatomy

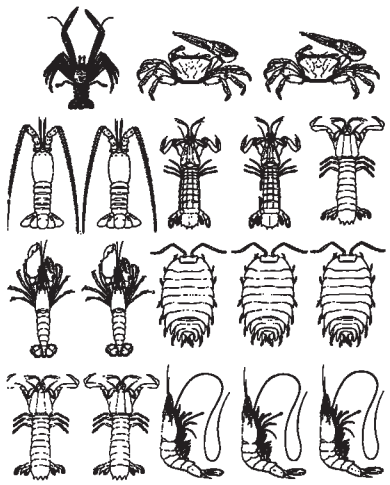


At first glance, few readers would associate this figure with Indian hemp, cannabis or *Cannabis sativa.* It shows, however, the anatomy of parts of this plant (for example, the epidermis of a bract) and is included in a new reissue of an atlas of microscopy published "for use in the identification and authentication of some plant materials employed as medicinal agents" (*Powdered Vegetable Drugs* by Jackson and Snowdon; Thornes, London, 1974, £4.00).

type viruses but grows preferentially in cat and primate cells and not in mouse cells. It remains to be elucidated whether this xenotropic murine virus is identical to the immunologically-activated virus. The presence of a transplant from a permissive host in an immunosuppressed recipient provides ideal conditions for the proliferation of xenotropic viruses.

It can hardly have escaped the attention of any of these investigators that human leukaemia viruses, if they exist, might be activated by chronic immunological reactions or by immunosuppression. Such a virus could account for the increased incidence of lymphoreticular tumours found in patients suffering autoimmune disease or carrying organ transplants. Since nobody has yet cried "Eureka", one must assume that mixed human lymphocyte cultures do not activate leukaemia viruses as readily as those of BALB/c mice. This is perhaps not surprising since Hirsch *et al.* found that the immunological activation of murine leukaemia virus could not be observed with all mouse strains. Furthermore, if the activated human virus were also xenotropic, its discovery may depend on finding the appropriate foreign cell type in which to grow the virus to detectable levels.

Crustaceorum Catalogus



THE Pinnotheridae (Decapoda, Brachyura)—the crustacean family whose members are commensal or parasatic—are on file in the latest part of the *Crustaceorum Catalogus* (part 3, edit. by Schmitt, McCain and Davidson; Junk, The Hague, 1973, 76 Dutch guilders). The catalogue is basically a compilation of the references contained in the pinnotherid sections of two card files started by Schmitt in the Smithsonian Institution, Washington. All pinnotherid references to 1968 and a few more recent publications are included.

Alpha clustering inside nuclei

from our Nuclear Theory Correspondent

MANY different lines of evidence indicate that the nucleons in the nuclear surface tend to group together to form α particles. The cross sections for the elastic scattering of α particles by nuclei and of α-transfer reactions require a significant degree of α clustering. Hartree-Fock calculations of the structure of light nuclei show a pronounced tendency to form groups of four nucleons that can be interpreted as α clusters.

A new approach to the problem of α clustering in nuclei has recently been made by Brink and Castro (*Nucl. Phys.* **A216**, 109; 1973). They were primarily interested in finding out whether there is any tendency to α clustering in the interior of heavy nuclei, so to simplify their calculations they considered infinite nuclear matter so that all surface and Coulomb effects could be neglected.

Two types of infinite nuclear matter were dealt with: one consisting of nucleons represented by plane waves and the other consisting of groups of four nucleons (α clusters) in a regular crystalline array. If a particular form of the nucleon-nucleon interaction is assumed, the total binding energy of the system may be calculated by standard quantum mechanical procedures. It is then possible to see whether the array of α particles is more stable than the nucleons and this in turn has implications for the structure of finite nuclei.

In such a calculation the results must not depend too critically on the assumptions made, so Brink and Castro used two extreme types of nuclear force (the Brink-Boeker and the Skyrme) and three types of crystalline array (the simple, body-centred and face-centred cubic lattices). In each case it was found that for the nucleon densities corresponding to those in the centre of heavy nuclei the nucleon matter was more stable than the α-cluster matter. This suggests that the α clustering inside heavy nuclei is rather small.

For a series of different nucleon densities, it was found that when the density is reduced to about one-third of that inside heavy nuclei the α-cluster matter becomes more stable. Thus as the density of nuclear matter is reduced there comes a point where it condenses into α particles. This is to be expected from the high binding energy of the α particle and the condensation takes place as soon as the density is low enough to prevent the interactions with all the nearby nucleons from breaking up any incipient clusters that may be formed.

The actual density at which the condensation occurs naturally depends on the nuclear force and on the form of the crystal lattice assumed in the calculation; but since the ones used were deliberately chosen to be rather extreme, any other realistic force or lattice will give results similar to those already obtained. Thus the general conclusion is not affected by the details of the assumptions made in the calculation.

It is natural to apply these results to the nuclear surface, where the density falls rapidly to zero, and to infer that in the outer region where the density is low some degree of α clustering will occur. This argument is very plausible qualitatively, although the complications of a finite system with a curved surface and the presence of the Coulomb interaction alters the value of the critical condensation density. Nevertheless this work has shown in a simple way that we may expect to find some degree of α clustering in the nuclear surface, but not in the interior.

Skeletonising the polypeptide

from a Correspondent

PROTEIN crystallography has progressed so rapidly in recent years, and so many new structures have been solved, that speakers at current meetings in the field generally give little information on how their results are actually obtained, that is, the pure mechanics of the business. They rather prefer to persuade the audience of the specific and general biochemical significance of their latest elaborations, beautiful atomic models in wire or ball-and-stick, photographed and presented stereoscopically. For the more traditional crystallographer in the audience, well aware as he is of the overriding biological aim of protein work, the implication that his métier is, though important, somewhat humdrum and routine, strikes a hollow note.

It is refreshing, therefore, to come across new papers which re-emphasise the enormous difficulties still to be overcome in the analysis of a structure as complex as a protein crystal, and which introduce new techniques for tackling these difficulties. Increasingly, computers have been used, and, if heavy-atom derivatives of the protein can be prepared and suitable automated X-ray diffraction apparatus is at hand, then there are many programs available to prepare the data and calculate the three-dimensional Fourier synthesis of electron

density. The problem that remains, that of assigning a polypeptide chain to the electron density, can involve many months of painstaking model-building work, and has hitherto been relatively unassisted by computer methods.

An important mechanical aid in model construction incorporates a half-silvered mirror, placed between the model and a plot of the electron density on perspex sheets, and arranged at such an angle that the model and the map appear superimposed to the viewer. This simple but ingenious device, called after its inventor, Professor Frederic Richards of Yale University, the Richards' box (by those in the trade, it is known more familiarly and affectionately as 'Fred's folly') has proved an indispensible aid. Even so, much experience and knowledge is required by the crystallographer to unravel and interpret the electron density map, and the prospect of programing a computer to take over this human task is daunting. Nevertheless, Greer (*J. molec. Biol.*, **82**, 279; 1974) has presented a method which goes a long way towards the solution of this problem.

Often, the amino acid sequency of the protein is known and this greatly assists the interpretation of the electron density map. The more clearly recognisable residues in the map may be correlated with the sequence and the more obscure ones inferred by extrapolation. To make the situation more general, however, Greer eschews the use of sequence information. Instead, he aims to produce a skeleton outline of the polypeptide chain by progressively chipping away at the density map.

The electron density map, of resolution between 2 and 3 Å, is plotted on a Cartesian grid of spacing about 1 Å. Certain criteria are established for the selection or rejection of points on the grid. A point is removed if its density is in the interval being examined in the current 'pass' through the map, and if no 'hole' is created by its removal. To test for the latter condition, the relative densities of the 26 nearest-neighbour grid points were examined. Further prerequisites for removal are that the point should not be at the 'tip' of a density region (for example, at the end of a side chain) and that the removal should not destroy the continuity of the skeleton (this requires a search of nearest-neighbour relationships).

An illustration of the application of these rules is given for a simulated two-dimensional map representing a polypeptide of thirteen residues. In order to isolate the main chain, the side chains, defined in each case as the skeletal piece between a 'tip' and 'branch point', are removed and their positions flagged. An exception to this procedure has to be made at the end of the main chain, defined by the coincidence of two side chain branches to a single branch point, since it is not known at this stage which is the main chain and which the terminal side chain. Main chain cross-bridges, due either to disulphide bridges or to hydrogen bonds are recorded.

The next major problem is to isolate a single molecule from its neighbours. The boundary is often poorly defined in the map and there may be considerable interpenetration of adjacent polypeptides. To eliminate unwanted chains, the symmetry operators and unit cell translations of the crystal are applied to each point or group of points to check whether it occurs more than once in the volume defined for one molecule. The redundant points are then chosen as those furthest from the centre of gravity of the molecule. There may still be unwanted pieces of neighbouring molecules present after this procedure, and the program searches for and eliminates all small chains that have both ends more than about 4 Å from any point on the skeleton. To reinstate portions of the main chain that may occasionally be deleted by mistake, the skeletons before and after the above procedures are compared, and the chain is examined for continuity.

The skeleton now represents the main chain punctuated by main chain branch points and side chain markers. These junctions indicate the positions of some of the α-carbon positions, and others can be found by invoking the so-called 4 Å rule, that the separation of residues along a polypeptide chain is always about 4 Å. Greer is developing methods for further automation of the map interpretation, but meanwhile the existing program already provides a head start for further model-building work by conventional techniques.

The program is applied to the 2.0 Å map of ribonuclease S, for which the structure is well established. A certain amount of manipulation is required to optimise its performance, but the net result is a set of residue positions which strikingly resembles that achieved by conventional methods. The mean deviation of measured and predicted α-carbon positions is 1.37 Å, which indicates good agreement since the skeleton was calculated on a 1 Å grid. The skeleton shows features such as the β-structure regions and the disulphide bridges clearly, but there are some trouble areas. Occasionally, three, four or five-membered rings occur instead of a straight chain, but these features are easy to detect and eliminate. Sometimes small pieces of chain are missing where the density of the map is low.

The comparative layman may easily be misled by the elegance and apparent ease with which computer techniques can produce seemingly convincing structural models of dazzling complexity, and it is necessary to be especially critical of the predictions made. In this paper

Sunspots affect Indian monsoon

Evidence that the solar cycle produces a marked effect on the weather on Earth continues to accumulate. King's recent study (*Nature*, **245**, 443; 1973) gathered sufficient evidence that there could remain little doubt of the reality of the effect; a report by Jagannathan and Bhalme emphasises that this effect is a major one, altering the circulation patterns of the terrestrial atmosphere significantly.

This evidence comes from a study of variations in south-west monsoon rainfall in India between 1901 and 1951 (*Mon. Weath. Rev.*, **101**, 691; 1973); the polynomial trend analyses and power spectrum analyses used reveal "composite patterns corresponding to the epochs of sunspot maximum, sunspot minimum, sunspot increasing and sunspot decreasing". The authors mention that 1899 was a year of severe famine on the Indian plains, brought about by extreme drought conditions, and that 1917 was a year of very heavy rainfall. No doubt students of the link between solar cycle and the weather will be intrigued to note that these were years of minimum and maximum solar activity, respectively; although Jagannathan and Bhalme do not specifically mention the present serious droughts in India, it seems difficult to avoid the conclusion that these may therefore be linked to the present period of low solar activity.

That would certainly fit the "composite patterns" noted; it seems particularly unfortunate that the link and these patterns were not discovered sooner, when they might have provided forwarning of the present situation. But it is unlikely that such warnings would have been taken seriously a few years ago; it is to be hoped, however, that research into these climatic variations will be accorded the recognition which the progress now being made justifies.

the firm results are the proposed α-carbon positions, and the correspondence of these to the known positions of ribonuclease S is convincingly demonstrated. It will be exciting to see the method applied to a completely new and unknown polypeptide or protein.

New structure, new mechanism for trypsin

from a Correspondent

IT may well be true that protein and enzyme crystallography has passed its zenith. But many laboratories engaged in this activity still continue to produce results that are often interesting, and even important. As the number of structures unravelled continues to increase, particular emphasis is being placed on structure–function relationships as well as on correlations between members of enzyme families.

The serine proteases are all enzymes involved in peptide bond cleavage during the processes of digestion, and the structures of two of them, chymotrypsin and elastase, were determined by X-ray analysis several years ago. Stroud and his colleagues now report the details of their studies on inhibited complexes of a third such enzyme, trypsin. Inhibition of trypsin by diisopropylfluoro-phosphate proceeds irreversibly, to give a complex, DIP-trypsin, and Stroud, Kay and Dickerson (*J. molec. Biol.*, **82**, 185; 1974) have determined its three-dimensional structure, initially at low, and then at higher (2.7Å) resolution. It is notable that the overall folding of the DIP-trypsin molecule is topologically very similar to that previously observed in chymotrypsin and elastase; the overall identity of sequence between these three is only 24%. The active-site architectures are likewise closely related. DIP reacts uniquely with Ser 195 and the position of the γ-oxygen of this residue corresponds closely to that in acylchymotrypsin.

Trypsin is also specifically and reversibly inhibited by benzamidine, in a way that probably mimics arginine side chains in a specific substrate. Krieger, Kay and Stroud have also determined the structure of this complex, which they describe in an accompanying article (*ibid.*, 209). The siting of the benzamidine in the enzyme-binding pocket, has induced alterations in the geometry of this pocket so that, in particular, it has undergone a marked closure so as to 'sandwich' the aromatic ring of the inhibitor molecule in between several peptide planes.

From the comparison at neutral *p*H of the structures of these two quite different inhibited derivatives of trypsin, Krieger *et al.* attempt to obtain information concerning the mechanism of the enzyme action. DIP-trypsin can be considered to be a model for the 'tetrahedral' intermediate widely accepted to be a covalent intermediate in the catalysis of serine proteases—whereas the benzamidine–trypsin complex may well represent a Michaelis complex (or indeed any other non-covalent intermediate, such as the often-suggested trans-conformation complex). Benzamidine is, in effect, a powerful competitive inhibitor for specific trypsin substrates, and it is quite reasonable to suppose that it binds in the same way as them. Moreover, the benzamidine–trypsin complex is stable at *p*H 8, when the enzyme is totally in its active conformation. Any observed change in the protein structure may then be solely attributed to the actual binding of the pseudo-substrate, and not to any shift in the balance between active and inactive conformations. The structure of active free trypsin remains to be solved, although Stroud *et al.* believe that DIP-trypsin should resemble it quite closely.

Insertion of benzamidine into the binding pocket involves the formation of a salt bridge with the β-carboxyl group of Asp 189, this residue accounting for the unique specificity of trypsin among the serine proteases for positively charged substrates. It is striking that this bridge is formed without any movement of the carboxylic acid, this residue being constrained by the tertiary structure. Any other substrate whose basic side chain has not the required length, will then, in forming the salt bridge, move the susceptible bond of the substrate away from its optimal catalysis position. The binding of benzamidine effectively induces certain changes in the active site, which in all favour rapid subsequent hydrolysis in the case of the specific substrates. The readjustment of the position of the most important residues of the enzyme when it binds a positively-charged substrate, may also explain the activating power of small amines for the hydrolysis of non-specific substrates.

It is worth noting that the charge-relay transfer mechanism involving the catalytic triad Asp_{102}, His_{57} and Ser_{195} proposed by Blow *et al.* (*Nature*, **221**, 337; 1969), for the acylation and deacylation of chymotrypsin, can also be applied to trypsin. Stroud *et al.* make use of the recent results obtained with α-lytic protease (an elastase-like serine protease), by Richards and his colleagues (*J. biol. Chem.*, **248**, 8306; 1973), using ^{13}C magnetic resonance studies with selective labellings in suggesting that the proton accepter is the aspartic acid, and not the histidine residue. The imidazole ring of this histidine, which remains neutral throughout catalysis, serves simply as a relay between the serine and the aspartic acid residues. It thus keeps the carboxylate in a hydrophobic environment such that its *p*K is increased to 6.7. The advantage of this new scheme (which is at variance with hitherto widely accepted doctrine), is that it implies no charge separation during the formation of the tetrahedral intermediate, and is therefore on the face of it, to be preferred energetically.

Limited progress with quantum gravity

from a Correspondent

MOST people would regard quantum theory and gravitation theory as two quite disjoint subjects; the first concerned with microscopic systems such as atoms, the second with objects of astronomical dimensions. Nevertheless, the marriage of these two unlikely bedfellows has fascinated a generation of physicists, for although quantum gravitional effects are deemed to be forever beyond the scope of direct experimental verification, the aesthetic appeal of a theoretical unification of these disciplines has always been compelling.

One of the driving forces behind research into quantum gravity is J. Wheeler (Princeton University), who has been visiting Britain during the past few weeks. His visit coincided with a conference on the subject held at the Rutherford Laboratory on February 15 and 16, and organised by D. Sciama (University of Oxford). The large number of participants testified to the extraordinary degree of interest in Britain for what is beginning to seem a theoretical obsession. With the sort of optimistic statements made recently by workers active in the subject, the conference might have been expected to produce convincing evidence of definite progress. In fact, it succeeded in generating a deep scepticism.

C. Isham (King's College, London) began with a review of the various levels of theoretical sophistication available to the would-be gravity quantiser, each level having its particular brand of ambiguities and problems. The presentation of technical results by M. Duff (University of Oxford) only served to confirm the extraordinary lengths which are necessary in order to make even minor progress. Pessimism mounted with evidence supplied by J. Taylor (King's College) that if gravitation is to be described by general relativity, then apparently it would be unrenormalisable in the usual sense familiar from quantum electrodynamics. That is, not only do infinite quantities appear everywhere in the theory, but they appear an infinite number of times for even the simplest type of process. Quantum gravity, said Taylor, "is dead".

If that is so, the subject was promptly resurrected by the following day with a series of talks by the 'fresh start' advocates. Adopting the philosophy that if the problems encountered on one level of the theory are insuperable, descend to another and start again, attempts were described in which the very structure of space and time are called to question. R. Penrose (Birkbeck College, London), supplemented by his student, G. Sparling, gave an account of his theory of twistors, but left a strong impression that the resulting formalism, whatever its intrinsic interest, is still very far from reality. A lot of comment was heard about starting from nothing and pulling all of physics out of a hat, but with no conceivable experimental framework to restrain their imaginations, the fresh starters continued to give the impression of drifting through a fairyland in which anything goes.

In marked contrast, work being carried out at less sophisticated levels does seem to be making some, if limited, progress. M. MacCullum (University of Cambridge) reviewed developments in quantum cosmology, which is an attempt to quantise the Universe considered (artificially) as a system with a small number of degrees of freedom. Undoubtedly the main attraction of the conference was a presentation by the indefatigable S. Hawking, now published in *Nature* (**248**, 30; 1974) on exploding black holes. This was based on recent work which shows that when quantised fields are examined in a curved space background metric, not itself quantised, remarkable effects such as the spontaneous creation of particles out of the vacuum may occur. According to Hawking, creation processes of this type would cause microscopic black holes to disappear in a burst of radiation. Exciting through this prospect may be, no plausible physical mechanism could be discerned which might lead to such a dramatic effect.

Last but not least, A. Salam (Imperial College, London) gave a most entertaining evening talk about almost everything, including evidence that the proton may have a finite lifetime, with all the consequences that may hold for the physics of the early big bang in cosmology.

Wheeler's own distinctive solution to the quantum gravity problem was described in his distinctive philosophical style to a packed gathering at King's College, London on February 27. Fortified with the belief that the ultimate equations of the Universe would reveal themselves by being "so beautiful and compelling" as to be obvious, Wheeler inclines to the view that space-time is built out of an underlying 'pre-geometry' in much the same way that elastic media are built out of atoms. Only the most tentative steps have been made along this fascinating path, based on the notion that the underlying structure should allow description in the simplest possible terms—perhaps just the logic of a 'yes-no' decision. For John Wheeler, this is a jumping-off point into the realms of philosophy, for how is the decision to be made? The mind, which has always had an uneasy co-existence with the world of the quantum, occupies a central role in Wheeler's universe—a 'participatory' universe as he calls it.

Whether or not all this will eventually result in a type of unification of quantum theory with space-time or gravitation theory, that will satisfy the aesthetic tastes of the physicist, is anybody's guess. What does emerge from these discussions is first the rather practical point that the quantisation programme so far has very little to do with general relativity (the discussion is almost exclusively dominated by field theorists and particle physicists) and, second, that progress with a full theory of quantum gravity (or if necessary a restructured space-time) is unlikely to be achieved until much more understanding of quantum field theory at the less sophisticated level of the Hawking result has been achieved. But the last word should perhaps be Wheeler's: when asked whether a young student wanting to work on quantum gravity should be a relativist or a field theorist, he replied with careful deliberation that such a person would be better advised to find something other than quantum gravity to work on!

Regulating the amounts of RNA

from our
Molecular Genetics Correspondent

ATTEMPTS to define the systems which control transcription have for the most part concentrated on individual gene systems whose products can be identified. Yet equally important is the problem of how the cell adjusts the general amounts of synthesis of different classes of RNA in response to changes in its environment. That the relative extents of transcription of unstable (messenger) RNA and of the two classes of stable (ribosomal and transfer) RNA are set in response to growth conditions is well established in bacteria. The increased complexity of RNA synthesis in eukaryotic cells, together with the lack of mutants influencing the control of transcription, have made comparable studies more difficult with higher organisms. But in a study of mouse fibroblasts maintained in culture, Johnson, Abelson, Green and Penman now report (*Cell*, **1**, 98; 1974) on the amounts of different classes of RNA present in resting and growing 3T3 and 3T6 cells.

With both cell lines—each derived from the fibroblast but with different sensitivities to contact inhibition and therefore possessing different growth controls—the amounts of both rRNA and mRNA are greater in growing than in resting cells. With 3T3 cells, in conditions of growth, there is some 2.8 times more rRNA; with 3T6 cells, growing cultures display 1.6 times more rRNA than resting cultures. But the mRNA content increases to a greater extent with growth; in 3T3 cells the increase is 4.0 times and in 3T6 cells it is some 2.3 times compared with resting cells. Each class of cell therefore increases its mRNA content more than its rRNA content in response to conditions promoting growth, the extent of the increase being characteristic of the cell line.

When serum (containing fibroblast growth factors) is added to 3T6 cells maintained in the resting state, the cells enter a quasi-synchronous division, the first cells entering the S phase of DNA synthesis after about 11 h, so that the first divisions take place after about 24 h. During the transition from resting to growth state, amounts of both ribosomal and transfer RNA start to increase about 11 h after stimulation and then continue to accumulate in a strictly coordinate manner. But mRNA behaves differently, increasing much more rapidly; the ratio of mRNA to rRNA increases 50% in the first 6 h and ultimately doubles. The rate of protein synthesis follows the rate of mRNA synthesis.

These results raise several intriguing questions about the systems which regulate the general amounts of RNA in cultured fibroblasts. One obvious point is that, unlike bacteria which possess only one known RNA polymerase, in eukaryotic cells mRNA synthesis is catalysed by one enzyme in the nucleoplasm whereas rRNA synthesis is controlled by a different enzymatic activity in the nucleolar apparatus. At least two different enzymatic activities, located in different cellular compartments, must therefore be controlled if any changes are made at the level of transcription.

Although the content of mRNA doubles before DNA synthesis begins in the transition from resting to growth state, previous work has shown that the overall rate of synthesis of HnRNA (the nuclear precursor to cytoplasmic messenger RNA) does not increase by this time—this implicates some step in mRNA production subsequent to synthesis of the precursor as a control stage at which the level of mRNA may be controlled. And since the rate of protein synthesis increases in harmony with the increase in messenger content—that

is before the increase in ribosome content—ribosomes must be used more efficiently during this period; this is consistent with models in which the frequency of initiation of protein synthesis is established simply by the amount in the cytoplasm of messenger RNA.

Amounts of ribosomal and transfer RNA seem to be perfectly coordinated in fibroblasts, unlike the situation in bacteria in which the ribosome/tRNA content may vary over a ten-fold range. But since these two classes of RNA display different stabilities in the fibroblast, their coordinate amounts cannot be established at the level of synthesis; neither rRNA nor tRNA is stable in resting cells, where their half lives are different, and rRNA is stable in growing cells whereas tRNA is not—details of the stabilities of different classes of RNA, a critical parameter in constructing models for the regulation of their activity, are reported by Johnson *et al.* to be in preparation. But the apparent coordination of the relative contents of tRNA and rRNA must clearly require noncoordinate synthesis. And the general response of the cell to conditions which stimulate growth is to increase its capacity for protein synthesis, the level of mRNA relative to that of the ribosomes and tRNA itself increasing so that the cell makes a more efficient use of the protein synthetic apparatus. Establishing the mechanisms implicated in the control of and response to the different states of the cell represented by the division cycle is clearly fundamental to any understanding of cell development, emphasising the importance of studies such as these which cast a light on the general systems that regulate RNA production.

Role of terminal sugars in biological events

from a Correspondent

DURING the past few years glycoproteins have moved dramatically from the ignominious position they long occupied of taking up the last forlorn session on the last day of international scientific meetings, to the glory of fully fledged symposia. There is scarcely a biological phenomenon that has not been bombarded with every laboratory's collection of lectins. Another obligatory reagent for research at present is neuraminidase, the effects of which are legion: on the immunogenicity of tumour cells; on receptor sites for viruses, hormones and certain lectins such as wheat germ lectin which has weak affinity for N-acetylneuraminic acid residues or other lectins having sialic acid specificity, for example, from *Limulus polyphemus;* on cell–cell interactions; on lymphocyte transformation, and so on.

The clear demonstration of a causal relationship between these various events and terminal sialic acid residues of glycoproteins or gangliosides is unfortunately less commonly obtained, though notable exceptions are binding of myxoviruses and certain lectins that clearly recognise this sugar and bind to it. Another candidate is the interaction of the natural peptide, tuftsin with polymorphonuclear leukocytes (Constantopoulos and Najjar, *J. biol. Chem.*, **248**, 3819–3822; 1973). The enhanced phagocytic capacity induced by tuftsin is abolished when cells are first treated with neuraminidase so raising the possibility that membrane sialic acid is the receptor site.

Perhaps the most convincing demonstration of a direct role of terminal sugar residues in the control of biological activity comes from Ashwell and his colleagues, who have shown that neuraminidase treatment of a large variety of soluble glycoproteins (including hormones) redirects these components from a normal circulatory pattern to one in which they are quickly cleared from the blood stream by sequestration in the liver. Ashwell and his collaborators have clearly identified receptor sites present on hepatocytes that recognise another terminal sugar, D-galactose, which is exposed as such after removal of sialic acid residues.

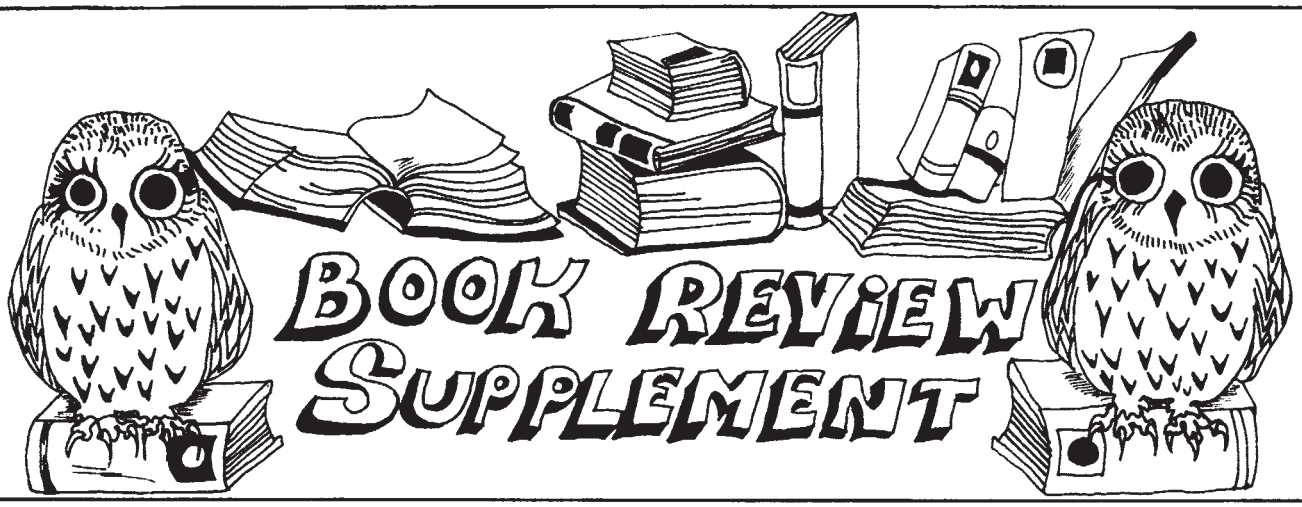
A possibly analogous situation is described by Sandford and her colleagues (*J. Immunol.*, **111**, 1071–1080; 1973) and may provide the explanation for earlier observations that the ability of a transplantable murine tumour to grow in certain foreign strains of mice is significantly reduced by prior treatment of the tumour cells with neuraminidase. Tumour cells treated with neuraminidase are rapidly killed by a naturally occurring antibody (probably 19S IgM) in the serum of resistant mice with specificity for newly exposed oligosaccharide terminals containing galactose residues.

The apparently widespread involvement of sialic acid terminals in biological activities, either directly or by virtue of a protective effect in 'hiding' other recognisable sugar terminals (galactose for instance), is a consequence of the ready availability of neuraminidases. Similar experiments using other glycosidases to point out possible functions of terminal sugars such as fucose have been hampered by the relative inaccessibility of active glycosidases of the required specificity. The recent report of Remold (*J. exp. Med.*, **138**, 1065–1076; 1973) suggests that it is only a question of time before other sugar residues are involved in biological events. Remold has studied the effect of the enzyme, L-fucosidase, on the interaction of macrophage inhibition factor (MIF) with its target cell.

MIF is a soluble glycoprotein of moderate size that is secreted by sensitised lymphocytes on exposure to antigen. It is so-called because of an ability to immobilise macrophages *in vitro*; macrophages are prevented in culture from migrating from the orifice of a capillary tube. The factor also performs other duties in enhancing the effector activity of macrophages in the immune response: for instance, in increasing their stickiness and phagocytic capability. It is relevant to point out that the carbohydrate unit of MIF seems to play a part in its activity since neuraminidase treatment inactivates MIF as shown 3 years ago by Remold and David. The unknown mechanism of action of MIF certainly involves as the first step, interaction of MIF with the macrophage surface. It is this step that Remold has now found to require intact fucose terminals on the glycoproteins of the surface membrane of the target cell.

First, guinea pig peritoneal macrophages, incubated *in vitro* with MIF in the presence of L-fucose at 100mM, fail to migrate as well as a control cell population and several other sugars have no effect. The inhibition by fucose is easily reversed by removing the sugar by dialysis. Second, when macrophages are treated with a specific glycosidase to remove L-fucose terminal residues from the surface, these cells do not respond to MIF but migrate normally. Clearly then the necessary fucose residues are part of cellular surface glycoproteins and are not part of MIF itself. Indeed MIF exposed to fucosidase in contrast to neuraminidase (or proteases) retains full biological activity. The inhibitory effect of fucosidase on macrophages is transient and the cells regain their ability to respond to MIF within a few hours, probably by replacement of the fucose-deficient surface membrane glycoproteins with complete molecules. The inference therefore is that the molecules containing receptor sites for MIF on the macrophage surface contain L-fucose residues and are manufactured by the macrophage population. Since L-fucose alone is inhibitory only at high concentrations, it seems likely that MIF recognises an extended oligosaccharide sequence terminating in an L-fucose residue in the receptor site on the macrophage surface.

The identification of this sequence and the question of whether it exists on a unique glycoprotein species of the macrophage surface, or alternatively if MIF will recognise a terminal L-fucose residue attached to any penultimate sequence remains to be determined. The competitive effect of L-fucose binding lectins on the activity of MIF would also be of interest and no doubt is under active study.



GENERAL BOOKS

Evolution and God

Darwin and his Critics. By David L. Hull. Pp. xii+473. (Harvard University: Cambridge; Oxford University: London, September 1973.) £9.25.

One might well ask whether a collection of the reviews of Darwin's *Origin of Species*, written shortly after 1859, could still be of any interest. Even a quick perusal of this volume answers this question in the affirmative; it shows how fascinating these reviews are and how amazingly pertinent to the present day. Even though written by scientists —contemporary reviews by clergymen are not included—they deal not only with questions of scientific evidence but raise a number of timeless problems such as the relation between science and a belief in the supernatural, questions of the correct method and philosophy of science, of the relations between biology and the physical sciences, and of the nature of scientific revolutions.

The Darwinian revolution occupies a unique position among scientific revolutions because, far more than any others, it caused a dramatic upheaval in the thinking of man. Only this can explain the bitterness, indeed sometimes downright nastiness, of some of the reviews. By his explicit analysis of all aspects of the diversity of the living world, Darwin forced every thinking reader into the uncomfortable position of having to make a choice in favour of one of the only three possible causal explanations of the living world: (1) continuing creation, that is the everlasting intervention of the creator in replacing species and faunas that had become extinct and in creating ever-new adjustments and adaptations; (2) a belief in the existence of evolutionary laws with a built-in teleology ordained at the time of creation, that would lead to ever greater perfection and adaptation, and to an orderly replacement of faunas in the geological sequence, and (3) a strictly non-teleological evolution, governed by random variation and natural selection, without any recourse to supernatural intervention, even at the beginning.

To accept the third of these interpretations meant rejecting the other two. Thus, the Darwinian revolution was not merely the replacement of one scientific theory by another, as had been the scientific revolutions in the physical sciences, but rather the replacement of a world view, in which the supernatural was accepted as a normal and relevant explanatory principle, by a new world view in which there was no room for supernatural forces. For the devout this meant a world without God, and such a view was heresy if not blasphemy.

Even though the present volume limits itself to sixteen reviews by scientists, the defence of the view that the world is the result of creation and governed by finalistic laws is prominent in each of the twelve reviews critical of Darwin. The reviewers are particularly stung by the silent implication of Darwin's thesis that it is unscientific to believe in supernatural causation. Hence their strenuous efforts to prove that Darwin himself is unscientific. Forever they repeat the claim that Darwin provides no proof for his manifold assertions, ignoring the fact that they were employing double standards, since Darwin's probabilistic evidence was immeasurably stronger than their own assertions of continuing creation. Rather remarkably, about half of the opposing reviews were authored by physicists, mathematicians, or other non-biologists. Their arguments often reveal an extraordinary ignorance of biological facts which they attempt to compensate for by accusing Darwin of having violated "the true method of science", that is a strictly inductive approach. Hull, a philosopher, treats this problem in detail in a seventy-five page introduction. He deals very perceptively with the role of philosophical concepts and methods in the Darwinian revolution, as they relate to essentialism, teleology, and the inductive method, and brings out impressively to what extent Darwin was ahead of his contemporaries even in these theoretical matters. Nothing could be more wrong than the immemorial assertion: 'Darwin was no philosopher'. Indeed, it was his philosophical rethinking which permitted Darwin to consider species as populations rather than as essences, a formulation that was the prerequisite for the adoption of natural selection as the directing factor in evolution. His empirical philosophy allowed Darwin to be satisfied with the probabilistic nature of his conclusions (against complete "certainty") and to adopt the hypothetico-deductive method.

One must grant Darwin's opponents the validity of two of their objections. First, Darwin produced embarrassingly little concrete evidence to back up some of his most important claims. This includes the change of one species into another in succeeding geological strata, or the production of new structures and taxonomic types by natural selection. More importantly, although some of this has become apparent only in retrospect, Darwin either left totally unanswered certain serious problems like the origin of life and of new genetic variation, or he was himself confused about the problem as with respect to speciation, to the nature of variation, and to the role of isolation.

How far Darwin was nevertheless ahead of his contemporaries is clear not

only from the opposing but also from the supporting reviews (Hooker, Carpenter, Fawcett, and Wright). Since they also rejected supernatural intervention, they felt that Darwin's explanation was by far the best available one. But even these supporters were, on the whole, not too comfortable with natural selection. Nor were they thinking truly in terms of variable populations. To shift over to Darwin's radically new thinking was obviously difficult for anyone who had been raised in an era of creationism and essentialism.

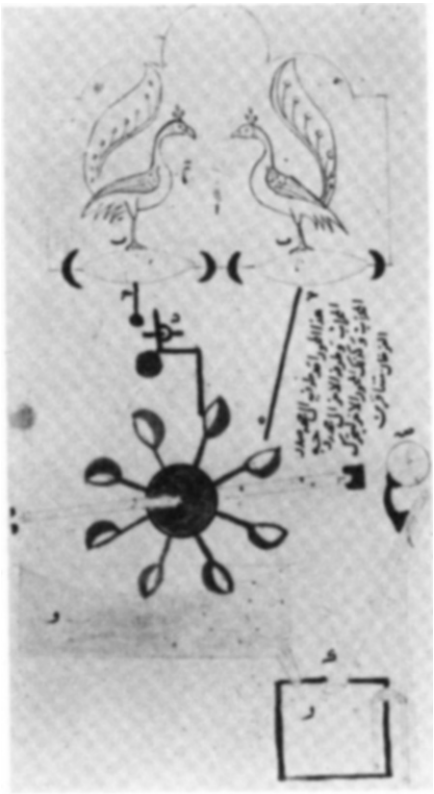
The length of the reviews astonishes a modern scientist. They average nineteen printed pages, even though the longest reviews (mostly of book length) by von Baer, Flourens, Kölliker, and Mivart are not included in this anthology (but short ones by von Baer and Mivart are). A book review in Darwin's day was an opportunity for the reviewer to write an essay on the subject matter of the book. Other reviews which were not included are listed in the bibliography.

The volume, in addition to reviews by eleven British authors, also includes five by foreign ones (Bronn, Pictet, von Baer, Wright, L. Agassiz). Curiously, Darwin was received much more favorably in America and Germany than in Britain. *Naturphilosophie* and a wave of extreme materialism had prepared for Darwin's enthusiastic reception in Germany. Unfortunately, however, Darwin was at once used as an ally in the fight against clericalism, particularly by Haeckel, and this rapidly led to a rather violent reaction, as reflected in the review by K. E. von Baer.

Hull has done a splendid job in editing these reviews. Each review is preceded by excerpts from Darwin's copious correspondence which deal with the reviewer, or more specifically, with the impact the review had had on Darwin. The editor then provides a concluding essay for each review in which the more important biographical details of the reviewer are presented, together with an analysis of his major arguments. The volume concludes with a detailed bibliography and a most helpful index. All in all an important and most stimulating contribution to the Darwin literature.

ERNST MAYR

Peacock water clock



IBN AL-RAZZĀZ AL-JAZARĪ culminated the Muslim engineering tradition of the twelfth century. One of his mechanical devices was this clock which, with its decorations ran on water power. Water from an ornamental pool fell into a tipping bucket which filled and tipped every half hour. Hence by a system of pulleys the time on the clock face was changed, a peacock and a peahen were rotated, and the peacock whistled. The illustration shows another part of the machinery: a water wheel which in turning caused two young peacocks to 'quarrel'. The crank in the left-hand arm means that the chicks were tilted together or apart by the turning of the wheel. Al-Jazarī's *Book of Knowledge of Ingenious Mechanical Devices* is translated and annotated by Donald R. Hill. (Reidel: Dortrecht and Boston, December 1973.) Dfl. 240; $96.00.

The political scientist

Liberation and the Aims of Science: an Essay on the Obstacles to building a Beautiful World. By Brian Easlea. Pp. xiv+370. (Chatto and Windus, for Sussex University: London, November 1973.) £5.50 boards; £2.50 paper.

OVER the years, well meaning scientists and educators have fostered various 'integrating' or 'bridging' studies of natural science; and they have subsequently been disappointed when the intended purveyors of wisdom revert to type and become specialised research workers. As the title of this book proclaims, the author, a lecturer at the University of Sussex, has retained the courage of his convictions. Some may take this book as evidence for the blessings of pedantry. Others may see it (and the course it summarises) as providing a healthy dose of politics to science students; it might convert them to radicals, or at least to more self-aware (and hence effective) servants of the system.

In structure the book is a very thick sandwich: rather more than a third in the middle is on politics, mainly contemporary. The beginning is a very good exposition of the Popperist-Kuhn debates, with well developed examples from the Copernican revolution and non-relativistic solutions to the electrodynamics crisis of the early twentieth century. Both these examples point up the role of judgement in the acceptance of scientific theories, and the impossibility of any simple formula for validation. In this aspect the author's philosophy of science moves towards Polanyi.

Having established "commitment" as central to scientific work, the author uses it to introduce his analysis of social philosophy. He briefly studies Adam Smith, John Stuart Mill, Karl Marx and Alfred Marshall from this point of view, showing that "commitment", while explaining much about a thinker, is not necessarily in contradiction with the "objectivity" of his analysis. Keynes and his ideas are studied at greater length, and the section concludes with an exposure of "objectivity" in the social sciences.

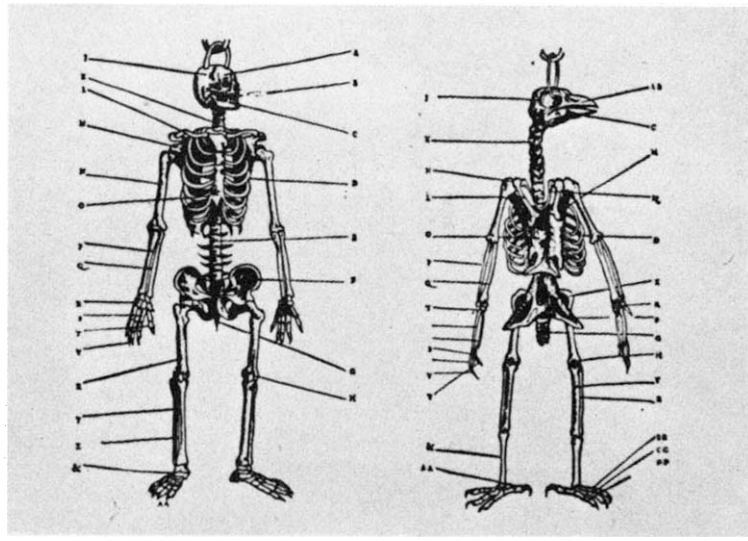
The argument then moves to society itself, providing a Marxist critique of American capitalism and neo-colonialism. There is a new contemporary relevance in the argument by Heilbroner (quoted here) that although imports from the "underdeveloped" countries to ourselves might be small in proportion to the total, they act as "vitamins" to the economy. Since we are unlikely again to be so casual and patronising about our indebtedness to the poor nations, it is salutary to be reminded how recently such an attitude was shared by all but the radical fringe. The political discussion concludes with "Socialism in Crisis", showing all the warts but blaming them all on the unfortunate persistence of capitalism. Had the author provided an anarchist critique of socialism to match his socialist critique of capitalism, this part of the book would have been much enriched.

The book returns to science for its final quarter; here the critique is mixed socialist and eco-romantic. Easlea gives a fine survey of the "counter-culture", and a reminder of moral weaknesses in the history of the atomic bomb. Very near the end of the book is an analysis of J. D. Bernal's essay of 1929, *The World, the Flesh and the Devil*; showing the marriage of reductionist and Faustian themes that formed the basis of his commitment. As a man of influence in his century, and a late representative of a tradition still dominant, Bernal provides a most useful example. By contrast, Easlea believes in a beautiful world where science would be play, following William Morris more than Karl Marx.

Easlea's humanitarian commitment, translated into a certain orientation in politics, is overwhelmingly strong. Yet it does not destroy his objectivity; he is generous to all but the most reprehensible of his opponents and freely admits weaknesses and difficulties in the causes he holds as good. Any scientist browsing through it (starting with the excellent resumé at the end, a feature to be praised and emulated) will occasionally find himself shocked and annoyed, sometimes lost in an unfamiliar area of the author's broad competence, but never bored and frequently stimulated. The imposition of intellectual discipline on an intense personal vision has succeeded in this case; those who know how hazardous an operation this is, can congratulate the author on his achievement.

J. R. Ravetz

Comparative anatomy



Drawing by Pierre Belon comparing the skeletons of man and bird. This appeared in his *L'histoire de la Nature des Oyseaux* and was the first attempt to link birds with mammals. Belon wrongly equated the collar bone of man with the coracoid bone of the bird. From *New Perspectives in Evolution*, by Querner, Holder, Egelhaaf, Jacobs and Herberer, translated from the German by Bonfield and Kamm. (Transworld: London, December 1973.) 80 p.

IQ and inequality

I.Q. In the Meritocracy. By R. J. Herrnstein. Pp. x+193. (Allen Lane: London, November 1973.) £2.50.

Inequality: A Reassesment of the Effect of Family and Schooling in America. By C. Jencks. Pp. xii+399. (Allen Lane: London, 1973.) £5.00.

Jencks and Herrnstein present two interpretations of the problem of inequality. Whereas Jencks is primarily concerned with the sources of individual differences in access to the educational, social and economic benefits of American culture, Herrnstein is more concerned about the less immediate consequences of biological inequality for social structure in a meritocracy. Both authors base part of their work on what they regard, individually, as acceptable models for variability in intelligence. To some extent their models differ, consequently their overall assessments of the role of intelligence also differ. Herrnstein, partly because he has adopted a model in which there is considerable genetical variability for IQ, affirms the social relevance of IQ measurements. Jencks, partly by his acceptance of a model in which genetical differences are less important, suggests that the contribution of genetical variation in IQ to traits that have social consequences is proportionately less than is sometimes supposed, although more than many would admit. The authors seem to differ in their interpretation of data, the veracity of which they both seem to accept. Such disagreement as exists therefore, stems not from differences in their attitude to the data, but from differences in analytical procedure.

Herrnstein does not attempt to analyse data. He has accepted a model based on Jinks and Fulker's biometrical genetical reanalysis of some of Burt's correlations for IQ. Although on this model some 80% of the variation in IQ may be attributed to genetical variation, a substantial part of this is due to the non-fixable contribution of dominance and to the increase in genetical variability arising from assortative mating. Jencks, on the other hand, provides a detailed reanalysis using the method of path coefficients and argues that the heritability of IQ is nearer 45%. In addition, he claims that different estimates of 'heritability' are obtained from different degrees of relationship and that there may be positive covariation between genotypic and environmental deviations: the so-called 'double advantage' phenomenon. Although Jencks suspects the presence of dominance, he does not treat the matter analytically, an omission which, as we shall see, could explain some of his unusual conclusions.

A fundamental weakness of Jencks's approach is his failure to make explicit the mathematical relationships between genetical paths in different pedigrees (path diagrams) and between different paths of the same pedigree. For example, not all the numerically feasible solutions given by Jencks in Table A-5 for the relationships between his paths g (between parental and offspring genotype) and h^2 (broad heritability) are genetically sensible. Genetical theory indicates that only solutions in which $g \leq \frac{1}{2}h^2$ for the parent-offspring covariation are genetically sensible. His failure to specify these restraints means that equal weight is given to sense and nonsense answers. When we consider the amount of assortative mating and make allowance for the possibility of dominance the only estimates of h^2 consistent with a genetical model lie between 0.59 and 0.76 corresponding with Jencks's estimates of g of 0.25 and 0.30. Rather than supporting a lower heritability, therefore, on this basis alone Jencks's data are consistent with the value obtained by fitting a biometrical genetical model to all the data simultaneously (see Tables 1 and 2).

A further weakness of Jencks's approach is his failure to deal systematically with dominance. This can be seriously misleading for a trait such as IQ for which there seems to be considerable non-additive genetical variation. Dominance could account for much of the apparent heterogeneity between estimates of heritability obtained from different degrees of relationship. The ratios given by Jencks do not estimate the broad (or narrow) heritability in the presence of dominance and the inconsistencies to which he refers do not reflect haphazard inconsistences in the data but the ordered departures from simple additivity which, within the limits of sampling variation, depend on the presence of a considerable amount of dominance (see Table 2).

Analytically, Jencks's procedure is inefficient. He estimates parameters from one set of data and uses these to correct estimates from other sets. This is not merely inelegant, it also yields the poorest approximations of the param-

really the case. But whatever else may be said about the quality of the data, their quantity is such that our estimates are fairly precise and our test of the model fairly sensitive.

Table 2 gives the weighted least squares estimates of the parameters. The standard errors are approximate and assume that the deviations between observed and expected are small enough to be ignored. The expected values obtained by fitting the model to the data are given alongside the corresponding observed values in Table 1. Although individual deviations are sometimes large, the overall weighted sum of squared deviations, which is χ^2 if the observations are normal, is small in relation to the total weighted sum of squares of the observations.

Although the two data sets differ with respect to μ and E_c they are consistent for the estimates of D_R, H_R and A. There is some support for Jencks's conclusion that the heritability of IQ is apparently lower in the American studies than in Burt's British study. The best estimate of the broad heritability of IQ scores, however, for Jencks's data, is 68% which is 50% higher than the estimate Jencks has accepted. Since the model fits his data we cannot support his conclusion that the data give a heterogeneous picture of the genetics of IQ. Neither can we conclude that the data provide any evidence of genotype-environment covariation when proper allowance is made for dominance. On the contrary, when we allow for such covariation in the simplest way by adding to our five-parameter model one further parameter, r_{ge}, to specify the contribution of this covariation, no significant improvement in fit of the model to the data set is achieved. Furthermore, the weighted least squares estimate of r_{ge} is -0.31 with an approximate standard error of 0.20. This is not only non-significantly different from zero but in so far as it is negative it has the opposite sign to the covariation postulated by Jencks in his model.

A small anomaly in the results of our analysis of Burt's data is that A is numerically (though not significantly) greater than μ. This anomaly is removed by stipulating that parents and offspring do not share developmentally important environmental features. The correlation between foster parent and adopted children then has to be accounted for partly by placement.

The analyses strikingly confirm Jinks and Fulker's conclusion regarding the importance of dominance variation. Even if we make allowance for possible overestimation this can best be explained only if dominance deviations at individual gene loci are large or if increasing dominant alleles are more frequent than their recessive counterparts. Coupled with the evidence for inbreeding depression, this suggests that IQ displays the pattern of genetical variation associated with a fitness character, that is, a trait which has been subject to a history of directional selection for increasing IQ score. Whatever else may be said about its social significance, IQ is clearly a trait of biological relevance.

Because much has already been made of the relatively low estimate of heritability argued for by Jencks, we have concentrated on the small part of his book devoted to this subject. We felt that it was important for the continuing discussion to establish that Jencks's American data do not in fact give a picture for the genetics of intelligence which differs in principle from that which has long been apparent from British studies. In so doing we have not done justice to the scholarly presentation and thought-provoking discussions contained in both Herrnstein's and Jencks's books, which have more in common than perhaps either author would admit to, and little that we would wish to dissent from. We are agreed that there are individual differences for IQ, that only a proportion of these differences might be removed by environmental manipulation, and that the features of the environment that would require modification to achieve this have still to be identified. Those concerned with inequalities in our society might just as well resign themselves therefore to the fact that individual differences for IQ at more or less their present level will continue to be an important factor. In these circumstances, those who wish to see progress towards a more equitable society should turn their attention to the means of ensuring that the greater social and financial rewards of our society are not reserved for those who, whether through their genotype or environment, have a higher than average IQ.

J. L. Jinks
L. J. Eaves

Plan by computer

Computers and Socialism. By Stephen Bodington. Pp. 245. (Spokesman: Nottingham, 1973.) £4.00.

The book considers, in broad terms, the impact that computers could have on society in the foreseeable future. Descriptions are in non-technical terms, and are aimed at the intelligent layman with an interest in computers and economics.

A chapter is devoted to an appraisal of modern computer technology. Although in the thirty pages involved one could hardly expect a complete survey, there are some omissions. Little is said of the difficulty in constructing large software systems, in spite of the fact that software now dominates hardware in cost terms. It is rapidly becoming apparent that our capacity to control such large systems is a limiting factor in the development of computers as a whole.

A major part of the book is devoted to the thesis that computers should permit better planning by provision of more accurate information. As is noted in the book, such changes are already taking place with the aid of linear pro-

Butterfly collection



Three of the 7,000 butterflies illustrated in colour in *Butterflies of the World* by H. L. Lewis. (Harrap: London, February 1974.) £10.00. *a*, Female *Parides plutonius*, widely distributed in Asia; *b*, *Idea hypermnestra*, found in South America; *c*, *Marpesia chiron*, from Australia and southern Asia. Not to scale.

gramming, PERT and even faster conventional accounting tehniques. Several computer-based management information systems are now being sold commercially. The success of such systems largely depends upon the presentation and analysis of the basic information provided. Bodington looks forward to accurate economic modelling as the major planning tool. He hopes that a wide dissemination of information from such models will permit a decentralised power structure, although the political mechanism for achieving this is not discussed.

A surprising omission, in view of the lengthy discussion of modelling methods, is that of the world models of Forrester and Meadows. The significance of such models could be greatly increased if their reliability could be improved. But it does not seem likely that economic systems can be predicted with any accuracy (as the wide fluctuations in the basic commodity markets tends to show).

The book is well produced, although some of the formula and computer material is not corretly set. The subject matter is well presented, and since it is one of the first books in this area, it makes a useful addition to a library. I hope, however, that more penetrating analyses of the impact of computers will soon be available.

B. A. WICHMANN

Archaeologists entertain

Archaeological Theory and Practice. Edited by D. E. Strong. Pp. xiv+308. (Seminar: London and New York, August 1973.) £5.50.

THIS book is a *Festschrift* offered by his colleagues on the staff of the Institute of Archaeology in London to its director, Professor W. F. Grimes, after his seventeen years of service in that position. It is not, therefore, a survey of the theoretical structure of archaeology, as its title might imply, but an agreeable and wide ranging anthology, reflecting the considerable scope of interests of the staff of the institute, from Roman London (where much of Grimes's own work was carried out) to islands in southeast Asia, and from Acheulian hand axes to mediaeval technology. A particularly welcome feature is the inclusion of articles on photogrammetry and photographic techniques by staff in the Departments of Archaeological Photography and Archaeological Drawing and Survey.

Among the themes underlying this wide breadth of interest, a concern for environmental archaeology is perhaps the most prominent. Two further contributions are of very wide methodological interest. Professor J. D. Evans, Grimes's successor as director, writes lucidly on his system of sherd analysis and sherd counts which he has used on the pottery from neolithic Knossos in Crete to very good effect, putting his findings on a firm quantitative basis. And M. H. Newcomer and F. R. Hodson describe a method here termed "constellation analysis", which permits the elegant comparison of different classifications of a given body of material, each classification being based on different criteria. A quantitative comparison is thus afforded of the results obtained from several independent approaches to the same set of data. In the example discussed the method has been applied to Newcomer's data on the burins from the upper palaeolithic levels at Ksar Akil in the Lebanon.

The human, almost anecdotal, interest of two contributions in particular catches the interest, for they are both at the same time contributions to scholarship. W. M. Bray, in "A page of *Punch*", reproduces a poem entitled "Monkeyana". This is a lampoon, published in 1861, of the participants in the great British Association debate held in Oxford the previous year, when Huxley clashed so memorably with Bishop Wilberforce. In his discussion Bray brings out both the warmth in the scholarly world of the evolution controversy and, in an illuminating way, the extent to which the debate was reflected in the popular press as a whole.

The second of these delightful disquisitions is by the editor of the volume, Professor D. E. Strong, whose sad and sudden death in Turkey was reported shortly after the publication of this book. Professor Strong always accompanied his wealth of scholarship in Roman archaeology with a very modern wit, and in "Roman museums" he considers the Romans as connoisseurs and collectors of ancient art with a very contemporary awareness. The parallel with the pretensions and preoccupations of our own times is never explicitly drawn, but lingers engagingly below the surface. As scholarly as it is entertaining, this is a splendidly original and human contribution to a varied and wide ranging book.

COLIN RENFREW

Maths of Ch'in Chiu-shao

Chinese Mathematics in the Thirteenth Century: The Shu-shu chiu-chang of Ch'in Chiu-shao. By Ulrich Libbrecht. Pp. xxxi+555. (MIT: Cambridge, Mass., and London, 1973.) £11.25.

"ORIENTAL mathematics may be an interesting curiosity, but Greek mathematics is the real thing" (G. H. Hardy, 1940). Hardy's words were written before Needham's *Science and Civilisation in China*. The book under review is an instalment of the volumes of detailed work needed before a successor to Needham, or at least a development of his mathematical section, can be attempted. But I don't think that either Needham or Libbrecht would have persuaded Hardy to change his views. The Greeks, lacking a decent notation for computation, developed mathematical ideas in a geometrical context. The ancient Chinese, having developed a notation of decimal place-values which allowed them to compute pretty efficiently and competently, developed their mathematics in a practical (though I know of nobody as practical as Archimedes) and pedestrian manner.

Perhaps changes here since Hardy's

In memoriam



GRAFTON ELLIOT SMITH (1871-1937) was a man of many parts; he was first and foremost an anatomist with a speciality for comparative neurology; later in life he became a determined and outspoken contributor to debates on man's physical and cultural evolution. These and the other interests of this extraordinary scientist, were reflected in the contributions to a symposium organised in November 1972 in commemoration of the centenary of Elliot Smith's birth by the Anatomical Society and the Zoological Society of London.

In the proceedings just published (*The Concepts of Human Evolution* (*Symp. zool. Soc. Lond.* No. 33) edit. by Lord Zuckerman (Academic: London, January 1974) £10.30; $29.00), Lord Zuckerman—one of the small group of anatomists alive today who both knew and worked with Elliot Smith—introduces the man; there follows a medley of other relevant contributions and the volume ends with a thought-provoking and controversial session on "Elliot Smith, Egypt and Diffusionism".

death throw some light on this matter. The development of the computer is tending to strangle mathematical thinking among all but the brightest of its users: so one should not be surprised that the computational efficiency of Chinese mathematics tended to inhibit mathematical inventiveness except in the case of brilliant individuals not part of the State's official mathematical bureaucracy. Ch'in Chiu-shao (probably 1202–1261) was one of four such now known to have lived, and worked independently, within half a century. The *Shu-Shu chiu-chang* is his mathematical legacy—a treatise with problems and answers intended for practical use. Ch'in, a soldier of fortune and administrator, was a colourful and dubious character who will doubtless, when Chinese mathematics is better known in the West, be included in the gallery of such characters with, for instance, such old friends as Cardano and Galois. The treatise is in nine chapters: apparently a significant structure already used in the *Ch'iu-chang suan-shu*, the standard textbook which in Ch'in's time had already been in use for about a thousand years (less than the time during which Euclid dominated the West!). The contents of Ch'in's book are in many ways similar to the earlier one, but the problems are harder and the fruit of a considerable individual development. But there are some things not to be found in the earlier work. For instance an algorithm for solving the so-called Chinese Remainder Problem (for which Ch'in is at present best known) is the most important. There is also what Dr Libbrecht, doubtless correctly, takes as clear evidence that Ch'in had completely anticipated Horner's method for the solution of equations. Ch'in was apparently so pleased with this method that he deliberately and unnecessarily complicated the solution of one problem in order to demonstrate his virtuosity with an equation of the (unnecessarily high) tenth degree.

Ch'in's book was intended, as the conventions of Chinese mathematics at the time required, as a practical treatise and not as a mathematical text as we know it: it was rather a cookery book, but the recipes were subtle and based on much deeper mathematical thinking than was expressly used in the text. It is for this reason that Dr Libbrecht's research into the book and its background is so valuable. At first sight Libbrecht's book is not an attractive work: it is written as a serious and scholarly study, not for easy reading, but to serve as a start for the accumulation of the knowledge necessary to write a more easily read work. But the book grows on one with deeper acquaintance; its integrity and thoroughness are totally convincing. Clearly Dr Libbrecht's second career as an oriental scientific historian (his first was as a teacher of mathematics) is destined to be brilliant and rewarding. The chapter on the socioeconomic information to be derived from Ch'in's book raises high hopes of interesting future developments.

D. B. Scott

Sussex replies to Rome

Thinking about the Future: a Critique of the Limits to Growth. Edited by H. S. D. Cole, C. Freeman, M. Jahoda and K. J. R. Pavitt. Pp. 218. (Chatto and Windus (Sussex University): London, 1973.) £3.00.

Has the development of the energy crisis in the past few months strengthened the argument of Meadows's *Limits to Growth*, and weakened the trenchant critique of it, *Thinking about the Future*, by the University of Sussex Science Policy Research Unit team?

The fact that oil reserves are finite has no direct bearing on the Arab use of the oil weapon. It has depended simply on Arab states controlling a proportion of existing production capacity large enough to control the market. The long lags in exploration and drilling and the provision of transport elsewhere has made their action effective in the short term. It has at a stroke, and by design, raised oil prices to the level where as Surrey and Bromley point out in chapter 8, alternative sources of energy become economic. They further remarked, writing early in 1973, "In addition to the political uncertainties surrounding the future of Middle Eastern oil, some of the small Arab states which are large oil producers may decide to conserve their oil reserves until prices rise, rather than accumulating large foreign exchange reserves which are subject to depreciation by inflation and major currency re-alignments".

Peccei, the founder of the Club of Rome which sponsored *Limits to Growth*, has said that the political factors omitted from the study exacerbate the problem, and these political factors have brought forward the threat of doomsday. But it is equally possible to argue that political factors, as one aspect of the flexibility of human response, have brought forward action on energy supplies in time to deal with supply limitations before they became physically acute. Politics, for all its frustrations, can make society more adaptive than its dynamic systems model, not less.

Likewise within the United Kingdom, whatever the political and economic consequences of the miners' industrial action in the short term, the long term effect can be to secure a more even distribution of wealth and power within one industrial society, thus averting the build-up of greater tensions in the future.

This is not to argue that all is for the best in the best of all possible worlds. But it does raise the question whether on population, food, resources, and pollution *The Limits to Growth* addressed the right problems at all.

The Sussex team, taking *The Limits to Growth* at its own valuation, reviews the evidence used and the structure of the model, and concludes they do not justify the conclusions drawn. As a bonus they put *Limits* in its ideological perspective, against the background of the history of economic thought, the movements of environmentalism and technocracy, and the processes of social change. On evidence and ideology the critique is strong. Experts in particular fields will disagree about the realism of some of the assumptions tried by the Sussex team but they will also criticise those made by Meadows. On the methodology of modelling systems of uncertain, tenuous and changing structure however, the Sussex critique is less sure footed. *The Limits of Growth* is deficient in having ignored the hard won experience of econometrics, stochastic estimation and control theory, and more general adaptive systems. Forrester and Meadows quite fairly argue that this systems apparatus as it stands cannot draw conclusions from the sparse information available on world environmental problems. But combined with a perception of the real world—Meadows's or anyone else's—it does give a more realistic approach to practical policy making than does the crudities of "systems dynamics", which is no more than the solution of simultaneous dynamic equations. The reason for this is that a wider systems theory illuminates the behaviour of indeterminate systems partially controlled with partial knowledge of system behaviour.

The Sussex critique does not touch this problem of adaptive behaviour beyond pointing to other factors in social change. There are these other factors, but their effect manifests itself in the indeterminacy, not the incoherence, of physical systems when viewed as systems on their own. But the physical systems are important enough to warrant the fullest analysis. For example the Sussex team is well equipped to analyse the evidence on the time lags and uncertainty in industrial innovation, a crucial factor in the ability of industrial society to adapt and survive. Perhaps without the inhibitions of criticising other work, but stimulated by the courage, even the sheer impudence, of *The Limits of Growth*, the Sussex team will contribute further to the vast task of building a genuine discipline of social systems analysis.

J. W. Bray

PHYSICAL SCIENCES

Splitting atoms

Nuclear Fission. By Robert Vandenbosch and John R. Huizenga. Pp. xii+422. (Academic: New York and London, November 1973.) $29.50.

FISSION is one of the most peculiar and important phenomena in the whole of nuclear physics. If the constants of nature had been slightly different it could not have occurred at all, and the history of the world after 1940 would have been completely changed. Yet in spite of its great practical importance it occupies a very peripheral position in nuclear physics itself. Concepts from the rest of nuclear physics are used to understand fission, but until recently studies of fission told us very little about the nucleus. By far the most important effect of fission on nuclear physics was indirectly through its applications. These led to the growth of vast industries and this so stimulated nuclear physics and made such large resources available that the subject is now several decades further advanced than it would have been if fission had never taken place.

One of the results of this situation was that although most people almost identified fission with nuclear physics, most nuclear physicists neither knew nor needed to know much about the fission process.

This situation has changed dramatically in the past few years with the discovery of a whole range of phenomena including shape isomers, intermediate structure and the double-humped potential barrier. These exciting developments have greatly stimulated research on fission and a much more detailed understanding of the process is now being obtained. From an entirely different direction the technical advances in heavy ion physics have made it possible to study the collision of heavy nuclei with each other, and thus to investigate the complementary fusion process.

The new knowledge has now consolidated to the extent that an extended survey is timely, and this book by Professors Vandenbosch and Huizenga fills the need admirably. Both authors have studied fission for many years and are in a position to present a detailed and authoritative account of present knowledge.

After a brief historical survey, they summarise the liquid drop and single particle models, and their application to nuclei of increasing deformation. The Strutinsky model combines the two by superposing the variations in nuclear properties from nucleus to nucleus due to their shell structure on the average behaviour given by the liquid drop model to give a detailed and accurate overall theory. This model is described in detail and applied to the systematisation of fission barrier heights and other features of fission.

Subsequent chapters consider the present state of knowledge on spontaneous fission, fission widths obtained from neutron resonance studies, the properties of low-lying levels of transition nuclei, the angular distributions of the fission fragments as a function of excitation energy and the competition between fission and neutron emission.

Fission can be induced by photons, deuterons, tritons and many other particles, and a study of the corresponding cross sections can give information about the fission potential barrier. Much is being learnt about the way a nucleus deforms on the way to fission, and the mass and density distributions as the separation takes place. The kinetic energy released in fission is of both practical and theoretical interest, as well as the distribution of mass and charge among the fission fragments. All these aspects are carefully described, and the three final chapters are devoted to the prompt neutrons from fission, ternary fission and the gamma rays emitted from the primary fission products.

Full references to relevant researches are given at the end of each chapter, enabling particular topics to be followed up in detail. This is a well balanced and comprehensive survey of our present knowledge of fission that fills a real need. It will be of great value not only to those studying fission itself but also to other nuclear physicists who find that due to its growing academic importance they now have to repair a conspicuous gap in their knowledge.

P. E. HODGSON

All people of the earth

Implications of Continental Drift to the Earth Sciences. Edited by D. H. Tarling and S. K. Runcorn. Vol. 1, pp. xvi+1–622; vol. 2 pp. xiv+623–1184. (Academic: London and New York, April 1973 and November 1973.) Vol. 1 £13.80; vol. 2 £13.00.

ON the face of it, Earth scientists can hardly be accused of failing to respond to social need, defined in this context as material demand. Geologists, geophysicists and geochemists have long been associated with prospecting and the discovery of minerals and fossil fuels; their brethren with eyes on higher things are now achieving ever greater success in the understanding and prediction of atmospheric phenomena, much to the benefit of agricultural and other pursuits; and a careful search might even show that something has been contributed to man's physical welfare by palaeontologists. But at the other end of the spectrum there is another, rather more glamorous and generally quite distinct Earth science community whose task it has been to elucidate the nature of the Earth for its own sake. In the purely intellectual sense the achievement of the latter group has been immense; to its various members we owe what has come to be known as the revolution in the Earth sciences and all that it implies in vindication of Thomas Kuhn's paradigmatic hypotheses. On a less esoteric plane, however, the material benefits which a society increasingly suspicious of science might conceivably expect from the scale of its investment have been somewhat less apparent.

For this last reason, the Newcastle upon Tyne (April 1972) conference on the "Implications of Continental Drift to the Earth Sciences" was, despite titular ambiguity and curious grammar, welcomed by some as a sign that the purer Earth scientists were beginning to perceive their social responsibilities. On the whole, however, this proved to be a naive expectation even though in other respects the conference was rightly pronounced a success. Something like 20% of the papers presented were devoted to background reviews of palaeomagnetism and sea floor spreading; about 35% dealt with the palaeontological, palaeogeographic and palaeoclimatic implications of these phenomena; and a similar preportion was concerned with geological implications in terms of continental and oceanic evolution, rifts and rift margins, and continental edge structures. What these figures and headings demonstrate on the credit side is that Earth scientists of all disciplines are now talking to each other to a quite remarkable degree, almost all apparently having accepted the general validity of the new global tectonics and the need to interpret both new and old data in those terms. But it is equally clear, at least as far as this conference was concerned, that few people are yet able or willing to see their work in terms of its natural resource implications. Of the 87 papers involved only six—a disappointingly low proportion—were specific enough to be classified under the general heading of "economic significance", although it could be argued with some justice that many of the more geologically inclined presentations form the essential base for subsequent, more applied studies.

The two books under review are a record of the proceedings of the New-

castle conference, and as such reflect both the merits and faults of the conference itself. At the same time, however, they present problems of their own. I was about to say that they are the academic equivalent of the book of the film, but on reflection it would be more true to say that what we actually have here in part is the book of the film of the book. In other words, no small proportion of the material presented has been published before in other forms.

It is time that the pressures—of tradition, commercialism, the ego, or whatever—to manufacture tomes such as these were resisted more often, not least because the result is self-defeating. It is quite reasonable, of course, that conference organisers should seek to make available to nonparticipants something of what the privileged few have found useful or enjoyable. But when such proceedings are produced incompleteness may be a virtue. It would have been far more profitable for everyone if Tarling and Runcorn had commissioned a small group of the best scientists and writers involved to distill the essence of the 87 original papers into a book of 200 pages or so, avoiding overlap, the bad, the indifferent and the peripheral, and perhaps even restricting the coverage. People, rather than merely libraries, would then have bought the book and read it. As it is, "so it must be, for now all length is torture". As Mark Antony might say.

PETER J. SMITH

Experimental X rays

X-Rays in Atomic and Nuclear Physics. By N. A. Dyson. Pp. xiii+380. (Longman: London, December 1973.) £8.

THIS book is the logical successor to Compton and Allison's *X-Rays in Theory and Practice*. The author emphasises the constant interest that has been maintained in the subject since the discovery of X rays by Roentgen in 1895, and points out that the succeeding years can be roughly divided into three eras, each twenty-five years long. The first of these, from 1895 to 1922, saw the laying of the foundations, and a multitude of experiments were performed with the prime purpose of elucidating the properties and behaviour of X rays. The second period, 1922 to 1945, was involved with the development of X-ray spectroscopy, and the design and construction of sensitive detection systems. The third period, from 1945 to the present day, has been concerned more with nuclear properties and with the physics of the stars. It is not often that a single branch of physics has kept up its momentum over such a long period and has, at the same time, been able to generate such important and wide-ranging offshoots as X-ray crystallography, Auger spectroscopy, and Mössbauer spectroscopy.

After a brief historical introduction, the author covers his chosen field in six chapters: the continuous X-ray spectrum; characteristic X rays; experimental techniques for the study of X rays; the absorption and scattering of X rays; X rays in radioactive decay; and some additional fields of X-ray study. There follows a number of appendices giving numerical data, a comprehensive bibliography arranged chapter by chapter, and finally a subject index.

The bias of the book is towards the experimental side which is understandable, since much of the physics of X rays is well understood and documented. The chapter concerned with experimental techniques is particularly notable for its wealth of detail for the construction of a variety of devices for the detection of both hard and soft X rays. Each chapter is copiously illustrated with diagrams emphasising the nature of the results that can be obtained in the particular area under discussion.

The last chapter is in some ways the most interesting one because here the author changes pace and selects various areas in which X rays have been used (such as various sorts of microanalysis), are being used (such as the isotope effect in absorption and emission), or are likely to play a dominant part (such as plasma physics and astrophysics). When, to all this, is added such important areas as medical physics and industrial radiography, it is clear why the author's mood seems to be so buoyant and optimistic. Certainly his claim that not only have X rays had a great part to play in the past, but are likely to continue to do so is certainly justified.

The book is economically written, well illustrated and carefully produced. It should be made available in all scientific libraries, and should find a place on the shelves of any individual who is interested in the production, properties, and applications of short wave-length radiation.

P. J. WHEATLEY

All dating is relative

Recent Earth History. By C. Vita-Finzi. Pp. xii+138. (Macmillan: London and Basingstoke, October 1973.) £3.00 boards; £1.50 paper.

THIS slim volume has an inappropriate title, for this relates rather to the author's original intention "to write an account of the physical changes undergone by the earth during Holocene times . . .", as he states in his disarming preface. This book presents the contention that chronology should be based upon dating and not upon stratigraphy, that is, that dating throughout earth history should be chronometric and not relative.

The author quotes the anonymous reviewer of an early draft of this book that "Palaeontology . . . and stratigraphy are humble and intellectually unexacting and nothing written by [the author] or anyone else can change this for a long time". Although obviously someone who knew very little of modern palaeontology and stratigraphy, that reviewer was probably correct in concluding that such a view would not be modified by this book's contents.

The author apparently understands that quantitative dating depends upon the resolving power of the particular method, but a failure to deal satisfactorily with Oakley's point that "All dating is in a sense relative", renders the book less than effective for its purpose. The dustcover's claim that the book is "clearly written and logically argued" is perhaps rather too obviously a commercial.

EDWARD A. FRANCIS

Many bodies

The Many-Body Problem. By W. E. Parry. (Oxford Studies in Physics.) Pp. x+217. (Clarendon: Oxford; Oxford University: London, December 1973.) £6.25.

DR PARRY, in writing this book, had a very clearly defined and limited aim to which he has stuck rigorously. Assuming that his readers would have a knowledge of basic statistical physics and of Dirac's approach to quantum theory, he provides them with a large number of the mathematical techniques currently used for dealing with many-body problems and shows how they are related. Applications are introduced to illuminate the mathematics but only for this purpose. Readers are referred elsewhere for a fuller study of specific physical problems. The result is a well balanced, comparatively slim book containing a weath of methods which include second quantisation, perturbation theory, Green functions, correlation functions, linear response theory, partial summation of diagrams, equations of motion, the drone-fermion representation in magnetism and the use of the variational principle.

Despite the large number of techniques introduced, the book has a uniformity which makes it easy to read and to relate one part to another. The point of departure (one I favour) is temperature-dependent perturbation theory for the grand partition function and other techniques are related to this. Expansions for the properties of the ground state are derived from this as a special case. This approach makes the

book particularly useful for solid state and liquid physicists but, perhaps less so for nuclear physicists.

The whole is characterised by great care and thoroughness. Motivation and the main line of attack are always made clear. Very rarely does a "let us consider" leap out at one from the dark. Despite this, Dr Parry does not shirk the difficulties. He provides full proofs of the various basic theorems including the sometimes tricky points but the mathematical completeness does not obscure the main argument. This makes the book, within its limit, self-contained. The exercises placed throughout the text enable the reader to test his progress. The approximations introduced, especially in the partial summation of diagrams, are carefully discussed. The result is a successful realisation of the author's aims and a book through the pages of which one's research students can safely be encouraged to roam.

G. Rickayzen

Prolific mathematician

The Art of Counting: selected writings. By Paul Erdös. Edited by Joel Spencer. (Mathematics of Our Time, vol. 5.) Pp. xxiii+742. (MIT: Cambridge and London, 1973.) $22.50.

This handsome volume contains a selection of about 80 papers from the prodigious output of Paul Erdös. It was produced to celebrate the 60th birthday of this peripatetic genius, and the selection has been made with a view to illustrating his unique contributions in combinatorics. In this latter respect the editor's choice is only partially successful (and the title is somewhat deceptive); for as Turan points out in a short introduction, combinatorial ideas have characterised Erdös's work in other fields too, and notably so in the theory of numbers. There is a glimpse of this in the papers concerned with latin rectangles, especially in the joint paper (314) with Chowla and Straus where a famous conjecture of Euler about pairwise orthogonal latin squares is shown to be just about as false as can be by means of quite deep arithmetical arguments. But this is not intended as a criticism but rather as an encouragement to other devoted friends of Erdös to set about collecting other portions of his work, not only as a tribute but also as a great service to scholarship.

A charming essay of reminiscences by Szekeres introduces chapter 1, which contains a seminal paper by Erdös and himself demonstrating for the first time the power of Ramsey's theorem; and this is also the theme of chapter 8. Chapter 2 contains four survey articles in which Erdös poses and discusses unsolved problems in his own inimitable style, and in the process charts the development of the subject. His matchless ability to generate problems and conjecture results, and his unselfish willingness to share his mathematical concerns with anyone prepared to have a go, have inspired his contemporaries, great and small alike, for many years; in these lectures we come as close as we can in print to his essential mathematical personality. There are chapters on the colouring of graphs, on extremal graph theory, on systems of sets, on block designs, on tournaments, on information theory and on random objects; in each there is food for thought for years to come.

There is no sample from the important work of Erdös with Rado and Hajnal on the partition calculus, but this work is to be the subject of an independent book. There is also reference to another forth-coming book, by Erdös and Spencer (the editor) on probabilistic methods in combinatorics; these play an important part in many of the papers and lead to some remarkable existence theorems. Erdös's ability to grasp the essentials of a subject, or a theorem, and to apply them in other contexts is well illustrated by the striking use he has made of probabilistic arguments in many fields.

Each chapter is prefaced by a short editorial commentary but there are few textual corrections and there could have been more information about the subsequent history of some of the problems that Erdös has proposed over the years; there is the occasional reference, but sometimes only to the author's name. At the end of the book is an invaluable bibliography of all of Erdös's papers up to the present—some 600 papers in all; it is quite clear what his target should be for his 100th birthday!

H. Halberstam

Gas chromatography

Techniques of Combined Gas Chromatography Mass Spectrometry: Applications in Organic Analysis. By William McFadden. Pp. 463. (Wiley-Interscience: New York and London, 1973.) n.p.

Gas Chromatography in Inorganics and Organometallics. By G. Guiochon and C. Pommier. Translated by STS, Inc., Ann Arbor, Michigan. Pp. viii+332. (Ann Arbor, Michigan; John Wiley; Chichester, December 73.) £10.

New Developments in Gas Chromatography. Edited by Howard Purnell. Pp. vii+408. (Advances in Analytical Chemistry and Instrumentation: vol 11.) (Wiley: New York and London, 1973.) n.p.

The combination of gas chromatography and mass spectrometry is the most powerful tool yet devised in the analytical chemistry of volatilisable organic materials, and there has been a need for a practically oriented book which would serve as a guide to the two techniques and their interrelation. McFadden's book seems to meet this need admirably. Both mass spectrometry and gas chromatography are introduced satisfactorily, but the technical emphasis is clearly stronger in the former so that the book will be most useful to those with some background and experience in gas chromatography. The interfacing of the two techniques, one vacuum and one atmospheric but otherwise beautifully compatible, is discussed in depth together with the methodology of operating them together and the role of computers. The main part of the book is based on a regular course of lectures and this seems to have given it an extra coherence and clarity. In a happy choice of method, the last chapter is a series of some sixteen short contributions by various authors each covering a different field of application or specialised technique.

The second book is essentially a translation of the excellent French text of Guiochon and Pommier on "Gas Chromatography in Inorganic Chemistry". Though the applications of gas chromatography in organic chemistry have been legion, those in inorganic chemistry are much more restricted. The whole field and its problems and successes are most clearly reviewed and considered in this work. After a short introduction to gas chromatography itself there follow discussions of analytical separations achieved in the fields of inorganic gases, halogens and non-metallic halides, metals and metal halides, hydrides, organometallic compounds, metal chelates and isotopes. Finally, there is a brief account of non-analytical applications such as the measurement of thermodynamic parameters and preparative chromatography. It is a pity that the translation is rather uninspired and the proof reading somewhat slipshod.

Purnell's *New Developments* is an interesting collection of seven specialist articles, most of which lie outside the realm of analytical chemistry suggested both by the title of the series with which this book is associated and indeed by the most common applications of the technique. The authority and personal emphasis which characterise most of the articles reflect the fact that they are written by recognised experts on their own fields. The first is a review of the many forensic applications of gas chromatography. The second provides an admirably clear introduction to the jargon and use of digital computers. Four articles then concentrate on the study of various processes of physicochemical interest by

gas-chromatographic methods; namely phase changes which occur in materials used as stationary phases, polymer structures and interactions (by inverse gas chromatography), the determination of the formation constants of complexes, and the kinetics of chemical reactions (by using the chromatographic column as a reactor). Finally, there is a timely, well balanced and dispassionate account of gas chromatography on the production (as distinct from the preparative) scale. This particular package may seem at first sight to have a rather narrow appeal, but I found it both satisfying and curiously homogeneous: it certainly provides a stimulating and very up to date introduction to the wider aspects of gas chromatography.

C. S. G. PHILLIPS

Physics with computers

Computational Physics. By David Potter. Pp. xi+384. (Wiley-Interscience: London and New York, October 1973.) £5.50 cloth; £2.50 paper.

THE widespread use of computers in problems of physical science is enabling investigations to be carried out which are not subject to some of the restrictions of mathematical analysis. As a consequence some, who have not given adequate attention to the difficulties of interpreting the voluminous output of computers, have consigned analysis to the back room. It is therefore pleasant to welcome a book which shows that good analysis and sound physical thinking must go hand in hand with numerical technique if computational results are to have any validity and reliability. The introduction, at least, should be compulsory reading for any physical scientist before he embarks on an extensive and expensive computer program. He will also find much that is well worth reading in the rest of the book but he must not expect to find a discussion of every problem of interest to him.

Although the book is called *Computational Physics* it really deals only with difference methods. There is no mention of finite elements or integral equations at all. Numerical analysis, as such, is confined to three chapters so that some topics are discussed more cursorily than desirable. Some places where fuller treatment is required are: interpolation and its difficulties; the fast Fourier transform; Poisson's equation when the boundary condition is not of Dirichlet type; eigenvalue methods. In general, the reader seeking the whys and wherefores of numerical techniques for ordinary and partial differential equations will have to consult other texts such as those of Lambert and Mitchell.

The book is not really suitable for undergraduate teaching, partly because there are no exercises and partly because so many ideas creep in unannounced; for example, spectral radius, tensor inner product notation, Poisson brackets, sodium chloride mesh (note also that the definition of spectral norm is unsatisfactory).

Nevertheless, postgraduate workers will find this an extremely useful book to have for several reasons. It collects together material on numerical attacks which is not readily accessible elsewhere and provides an extensive bibliography covering many fields of application. Throughout there is careful discussion of the physical and mathematical approximations undertaken in reaching a stage where the computer has a reasonable chance of supplying a satisfactory answer. The relative merits of alternative approaches are also described.

Chapters 5 and 6 are devoted to the motion of numbers of particles, with and without field averaging, as in the collisionless case in plasmas and astrophysics while compressible hydrodynamics is an example of collisions. Long range quantum forces appear in chapter 7. The distribution function approach and Vlasov's equation occupy chapter 8. Chapter 9, dealing with hydrodynamics is the longest. It shows how the particle-in-cell method can display the development of Helmholtz instability and the creation of a von Karman vortex street; the marker technique for moving surfaces and breaking waves is also given (though the absence of Zabusky's work on the non-linear interaction of waves is a notable omission).

The complex nature of compressible flow is clearly brought out, and, in chapter 10, the related problems of magnetohydrodynamics are emphasised. Evidently, some new good ideas are going to be necessary before the computer can cope adequately with the diverse phenomena of shock waves and other large amplitude disturbances.

Well printed, the book is good value and will be appreciated by researchers.

D. S. JONES

Life in glacial Mexico

Das Mexiko-Projekt der deutschen Forschungsgemeinschaft. 6: Geologische und paläontologische Untersuchungen im Valsequillo bei Puebla, Mexiko. By E. W. Guenther, with H. Bunde and G. Nobis. Pp. 177; 14 plates. (F. Steiner Verlag: Wiesbaden, 1973.) DM. 69.

THE Mexico project of the German Research Association is a regional study of the Puebla-Tlaxcala Basin, with man in his local environment, from the Stone Age to the present, as its central theme. The present work concerns the geology and palaeontology of a small area some 10 km south of the City of Puebla.

Bird's eye view



PHOTO-mosaic of the west coast of Schleswig-Holstein. Taken from *The Earth from Space* by J. Bodechtal and H.-G. Gierloff-Emden, translated by H. Mayhew and L. Evans (David and Charles: Newton Abbott, January 1974; £6.50), this is in fact one of the few illustrations in the book not taken from space. The importance of this kind of mosaic is that a series of photographs on a scale of about 1:20,000 can be combined and reduced in size to give a picture with scale corresponding to satellite photographs; it is just possible to bridge the gap between satellite photographs and ordinary aerial photography.

Valsequillo owes its importance, in this context, to finds of undoubted stone implements in association with remains of extinct mammals, at horizons which though still not securely dated, suggest dates of the order of 40–50 thousand years BP. If this is the case they might be of an age some 10 thousand years earlier than any other evidence for the presence of early man in the New World. The present work contributes new facts and considered judgments of their implications to the basic disciplines of geology and palaeontology, on which any final decision as to the date of the human occupation must ultimately rest.

The palaeontological finds, apart from those of equids and elephants, have been dealt with elsewhere by other authors, but their results are summarised by Guenther in the introduction. Carnivores represented are coyote, dire wolf, a sabre-toothed cat and a very large bear, *Arctodus simus*. One fragment of a tapir is the only perissodactyl other than the horses. Remains of a peccary, *Platygonus* cf. *compressus*, were found in several examples. Camelids were not uncommon and two species of *Camelops* and one *Tanu-*

The Solar Chromosphere

R. J. BRAY AND R. E. LOUGHHEAD

International Astrophysics Series

February 1974: 404 pages: 55 line illustrations and 40 pages of plates: 0 412 10730 9: hardback: £9.00

The subject of the solar chromosphere is central to modern developments in both solar physics and astrophysics generally, not least because modern observations suggest close links with such diverse phenomena as the solar wind and the deep-lying convection zone responsible for supergranulation.

This book forms a comprehensive discussion on the solar chromosphere and the many illustrations and tables give the reader easy access to modern quantitative data on the subject. The emphasis throughout is placed on the quiet chromosphere although a short chapter has been included on the active region.

Experimental Physics for Students

R. WHITTLE AND J. YARWOOD

December 1973: 370 pages: numerous illustrations: 0 412 09770 2: hardback: £4.95

This textbook describes in detail about 135 experiments suitable for undergraduate students. All the experiments described have been carried out in the laboratory and in all cases the necessary theoretical background is outlined before full descriptions are given of the procedure. In many instances, typical experimental results are provided and the analysis of the significance of these results is discussed.

A complete catalogue or further information on these titles, together with a list of stockists, is available from the publishers on request.

CHAPMAN & HALL, 11 New Fetter Lane, London EC4P 4EE

polama are indicated. Of pronghorn antelopes, there was a small *Breameryx* and two larger species, probably both *Tetrameryx*. Several finds of *Bison* sp., including a fine skull with horn-cores, are compared with known New World species. Edentates included a large armadillo (*Holmesina*), a single ossicle of a glyptodont, *Brachyostracon*, and the flattened pelvis of a ground-sloth, *Nothrotherium*.

Most of the remains came from the Middle (see below) fossiliferous horizon, perhaps datable to an interstadial of the Last Glaciation corresponding with the European Paudorf, 28,000–25,000 years BP.

H. Bunde gives topographical and geological maps marking the positions of more than 190 exposures visited, from which some 1,200 samples of loose sediments and 80 hard-rock samples were collected. Painstaking heavy-mineral analyses of these enabled him to divide the late Tertiary to Recent deposits into four main zones, according to their dominant heavy-mineral species: Pliocene, an Mg-hornblende zone; Pleistocene, a thick Fe-hornblende zone, interrupted near its summit by pumice and ash falls with dominant olivine, from a local vent. This included the lower fossiliferous deposits. The middle fossiliferous horizon lay near the base of the succeeding hypersthene zone, to which a Wisconsin date is attributed. Above, including the uppermost fossil beds, was a zone with dominant biotite, of Late Glacial and Postglacial date. The accompanying mineral spectra make these subdivisions perfectly convincing, and on their evidence were based correlations leading to a composite stratigraphical column in the above sense.

There is a clear potential practical value of this conclusion, for correlating generally very varied and only locally occurring fine-grained strata of differing appearance, often more or less weathered or redeposited by wind or water and in no other way readily assignable to their relative positions in a sequence. It may well have important results in the archaeological field when applied to deposits containing mammalian fossils and artifacts.

G. Nobis's study of the horse teeth recognised three groups: two larger species with primitive 'zebroid' features, the first of which might be assigned to *Equus* (*Hesperohippus*) *mexicanus*, the second remaining indeterminate. A third, smaller, species resembles *Equus conversidens* Owen, an asinoid parallel with the Eurasiatic *E. hydruntinus*. The specimens in question are well illustrated in two plates.

E. W. Guenther's contribution is an exhaustive study, with numerous graphs and scatter diagrams, illustrating the morphological and metrical characters of a collection of between 40 and 50 elephant teeth. He compares them with others found in Mexico and with the well known rich collections from Süssenborn (*P. trogontherii*) and Vogelherd (*M. primigenius*). He concludes that most represent *M. columbi*, about 10 of them *M. imperator* and only two, without provenance, *M. primigenius*. To these he adds a late, dwarf, species, hitherto undescribed, which he calls "*M. primigenius diminutivus* var. *mexicana*". The upshot is that practically all the elephants are of interstadial or interglacial forms, as the stratigraphy would suggest. The study is of methodological importance and introduces a number of new techniques for the measurement and graphical presentation of such material.

In all, this is an important publication, which should interest not only Americanists, but geologists and palaeantologists in general. The price, however, may restrict its availability to libraries only.

I. W. CORNWALL

Problems with light

Problems in Optics. By M. Rousseau and J. P. Mathieu. Translated by J. Warren Blaker. Pp. ix+366. (International Series of Monographs in Natural Philosophy Volume 52.) (Pergamon: Oxford and New York, 1973.)

THIS collection of problems in optics includes not only the classical aspects of physical optics, such as diffraction, interference, polarisation and dispersion but also electromagnetic waves, atomic and molecular spectroscopy and quantum mechanics. Although the problems set are keyed to the complementary text, *Optics* by Mathieu, in the same series, it is not really necessary to have access to this book to make good use of the present volume. The approach adopted is to set, usually three, closely related problems and to follow these by detailed solutions, each of which begins with a summary of the relevant physics required. There are many numerical computations so that the student is adequately drilled in the manipulation of units (MKSA rationalised units are used throughout) and is also given practice in estimating the orders of magnitude of the various quantities involved. The book can be fairly said to have met the two principal objectives of its authors, to teach the principles of optics and to teach the methods of problem solving. The text is well printed and the diagrams are clear and easily understood. The translation from the original French is excellent and reads well. There is good consistency in the use of symbols and constants with a convenient summary at the beginning.

With the renaissance in optics brought about by the developments in laser physics and space research, this book has come at an opportune time and should be particularly valuable to research workers in solving real problems, as well as to academic staff, who have to set examination questions, and to students who have to answer them. I have already found it invaluable in helping tutorial students to solve honours degree examination problems. To sum up, this is an excellent book and I strongly recommend it.

D. J. BRADLEY

Where the oil is

Oilfields of the World: Geology and Geography. By E. N. Tiratsoo. Pp. viii+376. (Scientific: Beaconsfield, 1973.) £8.50.

THE subtitle of this book, *Geology and Geography*, is an unduly large claim for a volume of only 376 pages. Nevertheless it is a useful and up to date reference book, the more valuable for its appearance at this critical time in the history of exploration of natural hydrocarbons.

The book begins with an introduction on the history, nature and occurrence of oilfields. Much of this is elementary, but it has the merit of defining and explaining many of the terms peculiar to the petroleum industry, definitions often not readily available to the non-specialist (for example 'naphtha', 'middle distillate', the niceties of varying reservoir type and of different ways of quoting reserves figures).

The greater part of the book is occupied by a catalogue of the world's oilfields. This is somewhat uneven in emphasis, with relatively modest space given to the Middle East in proportion to the bulk of its reserves (a tenth of the space for half the world's oil), but it has the particular merit of giving generous treatment of areas on which information is much more difficult to obtain—notably Eastern Europe, the Soviet Union and Eastern Asia. The treatment of the United States is perhaps disproportionately long (twice the space allotted to the Middle East) but the enormous number of small fields and the overall complexity would make any shorter treatment very difficult to arrange.

The main disappointment is that the treatment of geology is scanty in the extreme. The age and nature of reservoir beds is quoted for most of the oilfields described, but there are no stratigraphic columns, no structural maps, and no structural sections except for the generalised textbook diagrams in the introduction. With a great wealth of available material to draw upon in the world's literature, this seems a major opportunity lost, and the brief descriptions included in the text do not go very far to fill the lacuna. The net effect has been the production of a highly informative geography of petroleum sources, but it is a catalogue rather than a stimulus to ideas.

Participants in the recent Royal Society symposium on "Energy in the 1980s" may be interested to see that units are given uniformly in barrels per day or barrels per annum. There is certainly merit in uniformity, and the author has successfully avoided the pitfalls of different kinds of tons and of (for example) the three different official conversion factors for "coal equivalents". Nevertheless it would have been useful to have had summary figures given in energy terms, in joules or 'Q', for those users of the book who are attempting to relate mineral oil to the statistics of other sources of world energy.

The book ends with a chapter on reserves, requirements and future supply, in which the potential for expansion of conventional sources, and the alternatives provided by tar sands and oil shales are discussed. Dr Tiratsoo echoed the warnings of Warman in the United Kingdom, Halbouty, and others in the United States, that the rate of discovery of giant oilfields (the significant contributors to the world's reserves) has fallen sharply since 1960, and—writing before the current potantial crisis—he concluded by underlining the decision of Kuwait and Iraq, made in 1972, to curtail production against future requirements with the apposite quotation from the Rubaiyat:

"I often wonder what the Vintners buy
One half so precious as the stuff they sell".

The industry has not been devoid of people clearsighted enough to envisage the present crisis; it is a pity that governments have not always been equally prescient.

P. E. KENT

Background to a battle

The Quantum Theory of Light. By R. Loudon. (Clarendon: Oxford; Oxford University: London, November 1973.) £7.50.

THE quantum theory of electromagnetic radiation has once again become the subject of lively discussion, thanks to the advances in the field of laser physics. These advances have made easily available sources of radiation with coherence and intensity characteristics completely different from those used by experimenters in the past and have produced new controversies over the specific application of quantisation to radiation fields.

But before it is possible to enter the quite heated battles surrounding this subject, it is necessary to obtain a grounding in the basic theoretical concepts involved in the discussion of the interaction of radiation with matter. Dr Loudon's book provides an excellent beginning to such a grounding, at a level suitable for graduate students, or good finals-year undergraduates.

Beginning with a review of classical radiation theory as applied to emission and absorption processes, the classical theory of optical coherence is briefly discussed. Quantisation of the radiation field is introduced, together with the properties of photon phase and number operators. This leads to a discussion of photon absorption and emission in terms of quantised field theory and a general discussion of photon optics including a brief section on laser theory and nonlinear optics.

To briefly single out only two chapters: the discussion of classical coherence theory is very well done and the notation carefully chosen so that an easy transition to the quantised approach can be made. Similarly, the discussion of photon optics provides an excellent introduction to the subject.

The general production of the book is excellent. The salting of the text with problems, which might be thought inappropriate at this level, seems an excellent idea and the references at the end of each chapter are comprehensive.

Although I have definite reservations on the blanket use of quantum field theory in optics, this book can be highly recommended to students in the field.

D. G. C. JONES

Unimolecular reactions

Theory of Unimolecular Reactions. By Wendell Forst. Pp. xv+445. (Physical Chemistry: vol. 30.) (Academic (Harcourt Brace Jovanovich): New York and London, November 1973.) $29.50.

ANYBODY who has had a more than superficial interest in the theory of unimolecular reactions will be familiar with the names of Wendell Forst and O. K. Rice, who contributes the foreword. The latter's encouragement and stimulus to many research workers in this field, including Wendell Forst, should not pass without mention.

The volume is divided into two parts, the first of which deals with the general concept of the unimolecular process, the definition of such basic quantities as average rate and the basic assumptions involved. Slater theory is mentioned only in passing and to place its assumptions in correct prospective. There follows a discussion of intramolecular energy transfer and the assumption of energy randomisation. Chapter 4 considers the statistical cal-

culation of the unimolecular rate with respect to phase space in both classical and quantum-mechanical formats; pertinent degrees of freedom are thoroughly discussed, due attention being drawn to the necessary restrictions implied by conservation theorems. As might be anticipated, a lengthy and excellent chapter on energy-level densities is included and should enable the reader to utilise the various approaches in actual calculations.

Part 2 divides itself naturally, through the average energy of the excited species, into a discussion of thermal reactions, chemical activation systems (including photoactivation) and, most welcome, a detailed consideration of the fragmentation of highly excited molecules and ions. These make excellent reading indeed, and the detailed analysis of rotational effects, where appropriate, is certainly worthy of comment.

References are given at the end of each chapter and are both extensive and up to date; a glossary of the symbols used, together with their clear definitions (always welcome in this field), is given as well as an author and subject index.

The author rightly appeals in an appendix, for the more extensive use of the results of the steepest descents method of calculating energy-level densities. A simple basic programme is given as further encouragement.

I found my task of reviewing this book both rewarding and enjoyable, and even at a price in excess of twelve pounds, no one with any pretensions to interest in this field can afford to be without a copy.

W. J. Orville-Thomas

Arctic circle

Arctic Geology. (Proceedings of the Second International Symposium on Arctic Geology, held February 1–4, 1971 at San Francisco.) Edited by Max G. Pitcher. Pp. xvii+747. (American Association of Petroleum Geologists: Tulsa, Oklahoma, 1973.) $30.

There is a certain scale of geological phenomenon that may suddenly come into focus when the appropriate mixture of specialists come together at a symposium. The formula works particularly well when the solution of a geological problem requires knowledge of the geology of a number of countries. This book contains some seventy papers read at a symposium on Arctic geology held early in 1971. To my mind the result is particularly rewarding in two ways. The specialist will find discussions of the geology of Alaska, of Arctic Canada and the Soviet Union, of the Arctic ocean, the North Atlantic and the Northern arc of the circum-Pacific fold belts together with first class reviews of various stages in their geological history.

But above this and of more importance to the science as a whole the symposium succeeded in illuminating the geology and the economic potential of the succession of sedimentary basins which overlie the Precambrian blocks clustered around the Arctic Ocean. By the end of the century a quarter of the world's production of hydrocarbons may come from the Arctic. The ring of Precambrian blocks which underlie Canada, Greenland, Northern Europe, north-west and north-east Siberia have separated and come together in a complicated fashion during the past 500 million years. The mountains of Scotland and Scandinavia, the Appalachians, the Urals, the island arcs and the mountain chains around the northern Pacific and the North Atlantic itself are direct results of these movements. Their history is traced out in this book, as is the history of the sedimentary basins which are so important as potential or actual sources of petroleum. This book can be recommended as providing an excellent synthesis of a region likely to become of ever increasing importance in the remainder of this century.

John Sutton

Impurities have virtues

Deep Impurities in Semiconductors. By A. G. Milnes. Pp. xviii+526. (Wiley-InterScience: New York and London, 1973.) n.p.

Studies of the electrical properties of semiconductor crystals containing impurities fall into at least two distinct categories. The first is concerned with the determination of the position and nature of localised electronic states due to specific impurities, and perhaps the accounting for these properties in terms of theoretial models. The second category relates to the electrical behaviour of such material under non-equilibrium conditions and to the properties of junction structures. It is important to recognise that work in these two areas is in general carried out by different groups of research workers and consequently the bridge between them is often quite tenuous.

The present volume aims to be complete in this context, although analyses of defects produced by irradiation damage are explicitly omitted. The theory of localised energy levels is discussed briefly in chapter 1, which is followed by reviews of data for silicon, germanium, gallium arsenide, gallium phosphide and other III-V compound crystals in chapters 2 and 3. The latter chapters read very much as a catalogue, but are nevertheless valuable because of the large number of references cited. There is then a marked change in style in chapters 4, 5 and 6, which deal with theories of steady state statistics of deep impurities, trapping and recombination. Apart from a few examples, of which gold doped silicon is prominent, the deep levels are not considered in relation to particular systems. The emphasis becomes more practical in the following chapters with discussions of photoconductivity, recombination and trapping in junctions, thermally stimulated currents and techniques for measuring lifetimes.

The contents of the remaining four chapters are of considerably more interest. It is shown in some detail how the presence of deep impurities can lead to a negative differential resistance, and oscillatory effects are discussed at length. The relevance of the final chapter on conductivity by hopping processes to deep impurities is not too clear, as is pointed out by the author in the very final paragraph.

An overall impression gained from this book is that the difficulties referred to in the opening paragraph of this review have not yet been overcome, although of course this in no way reflects adversely on the text. A second impression is that the author has given prominence to the beneficial aspects of deep impurities, citing the value of infrared detectors and possible future uses of oscillatory phenomena. Adverse effects such as the quenching of luminescence, reduction in carrier mobilities and lifetimes would be regarded by many as being equally if not more important. Finally it should be noted that the very large bibliography containing nearly 1,900 items should be invaluable to workers in this field.

R. C. Newman

Ancient climates studied

Climatic Fluctuations of the Ice Age. By Burckhard Frenzel. Translated from the German by A. E. M. Nairn. Pp. xxiv+306. (Case Western Reserve University: Cleveland and London, November 1973.) $22.50.

The world's climate is not only changing, but changing on a timescale rapid enough to affect present day economic planning. Interest is growing in the problems of climatic change in general, and in such spectacular examples as ice ages in particular. So the arrival of this translation of Frenzel's 1967 German language study of the most recent ice age is particularly well timed.

The book concentrates upon facts, rather than hypotheses to explain the factual variations, and thus provides a useful reference for anyone wishing to



PINGOS in East Greenland. Squeezed between the permafrost layer below and the winter freezing layer above, the water-bearing horizon in some Arctic regions can be subjected to pressures of 50 atmospheres. This causes hillocks to form, up to 40 m high and 100 to 200 m in diameter; when the ice core of the pingo eventually melts, a crater-like depression is left, and these depressions may remain as a visible record of a period of severe climate.

jump off into the unknown with their own hypotheses. Climatic fluctuations during the Pleistocene undoubtedly have their origins in the same changes in the global pattern of circulation, or of input of solar radiation, that cause the climate to change today—but of course, it is not yet clear exactly what those changes are. This well planned and thorough discussion of the methods by which ancient climates can be studied, and what comes out of those studies, will be particularly useful to those people from many disciplines who are turning part, at least, of their attention to these questions; not least among the useful features of the book is the comprehensive bibliography.

JOHN GRIBBIN

How many worlds?

The Many-Worlds Interpretation of Quantum Mechanics. Edited by Bryce S. DeWitt and Neill Graham. Pp. viii+252. (Princeton University: Princeton, November 1973.) $12.50.

THE 'many-worlds' interpretation is surely the most exotic product of the endless struggle to understand quantum mechanics. To avoid any suggestion of the infamous 'reduction of the wave packet' it is supposed that not only does the wave function not reduce, during measurement, but that physical reality multiplies. On each occasion not just one but all possible measurement results are realised! To accommodate them all an increasingly multiple world is required, one 'branch world' for each possible sequence of results. It is supposed that, in each branch, objects (including people) interact with (and are conscious of) others in the same branch only. So we are not aware of the many worlds that we do not inhabit; the people, often very very like us, living in those other worlds, are other people.

If you have not personally found the time to grapple seriously with the puzzles of quantum mechanics it can hardly be intelligible to you that physicists might be driven to such lengths. And even if you have found the time, you may wonder in what way such a conception is superior to the de Broglie-Bohm pilot wave theory; there also the wave never reduces, but only one of the many possible physical configurations is realised, and evolves in time in a way consistent with quantum mechanical probabilities. My opinion is that the pilot wave provides a simpler and more economical solution of the problems motivating both approaches, and is quite superior in requiring neither any arbitrary classification of physical processes into 'measurements' and others, nor any arbitrary division of the world into 'systems' and 'apparatus'. I have to add, however, that my understanding of the pilot wave theory was much enhanced by some aspects of the 'many world' picture, in particular its emphasis on memory states. Moreover it is the very clarity and precision of the pilot wave theory which puts in prominence the particular difficulty concerning locality. But this is not the place to develop that theme.

The book under review is mainly a collection of preprints: the original 1957 paper of Everett, a contemporary assessment of it by Wheeler, later expositions by de Witt (1969 and 1970), and a later rediscovery of similar ideas about correlations in memory states by Cooper and Van Vechten (1969). Cooper and Van Vechten explicitly invoke 'irreversibility' to distinguish some physical processes from others as 'measurements', so exhibiting more prominently a basic vagueness of the approach. They also explicitly dissociate themselves, in a footnote added in 1972, from the many worlds concept; they see no reason for more than one of all the possible worlds actually to 'come to pass'. This puts them still closer to de Broglie and Bohm, but even they do not recognise this affinity.

In addition there are two hitherto unpublished papers. One is an alternative account by Everett himself, written apparently before the well known paper mentioned above. Although it does not add anything (it seems to me) on central points it contains some interesting auxiliary material, including in particular a discussion of various measures of uncertainty, and some comments on other approaches to quantum mechanics. Here the de Broglie-Bohm picture is mentioned; Everett thinks his own picture is simpler. The other new paper is by Neill Graham. He is concerned about the arbitrariness of the measure (actually the quantum mechanical norm) invoked to weight the various branch worlds. He argues that with sufficiently complicated (ergodic) apparatus simple equal weighting of different branches is practically equivalent. In the form actually given the argument supposes the multiple world divided into discrete branches, classified by the discrete eigenvalues of some privileged "observables". That suitable operators with discrete eigenvalues can be considered in the neat world of academic quantum mechanical measurement theory is clear. The messy real world, in which each piece of measuring equipment is immersed in a particle and radiation bath from which it is only vaguely separated (on the microscopic scale) may be another problem.

The search for a satisfactory interpretation of quantum mechanics is likely to continue for a long time among many, rightly or wrongly, unsatisfied people. The many worlds interpretation, its tenability or untenability, and the sidelights it throws on other schemes, will continue to be discussed. For participants in that discussion this will be a useful book to have.

J. S. BELL

Quantum textbook

Fields and Particles: An Introduction to Electromagnetic Wave Phenomena and Quantum Physics. By Francis Bitter and Heinrich A. Medicus. Pp. xiii+688. (American Elsevier: New York, London and Amsterdam, 1973.) Dfl. 52; $20.

THIS is an ambitious book which takes the reader from electromagnetism through a wide variety of wave phenomena to a discussion of the symmetries of elementary particles. For so large a book the price is very reasonable, and at its best its merits are sufficient for the buyer to feel he has got his money's worth. Nevertheless there are notable imperfections which cause me to hesitate before recommending it as the basis of a major course at, say, first or second year level in an English university.

To begin with a few minor examples, as typical of the rather numerous blemishes to be eliminated in the next edition: it is implied (page 199) that as a wave packet spreads by dispersion, the frequency range in its composition gets less; the discussion of Poynting's vector (page 627) involves a mathematical oversimplification which carries the corollary that all lines of force are straight; the phase-amplitude diagram

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T T Macan

A Review of the first edition:
"A much needed book, written by a foremost authority on the subject. . this book will be welcomed in every university zoology department and in sixth forms. . No ecologist can afford to miss it"
British Book News

Statistical Tables for Biological, Agricultural & Medical Research
Sixth Edition
Fisher and Yates

This book of statistical tables, first published in 1938 by Oliver & Boyd, is now being reprinted by Longman. This is the Sixth Edition (1963) with minor corrections. It has long been regarded as a standard work essential for all statisticians and biometricians, especially those concerned with investigations on highly variable material. £2.50



Longman 1724-1974

(page 90), so essential for an appreciation of diffraction at this level (and for that matter at a much higher level), is thrown in far too peremptorily for a student whose experience of complex numbers is as rudimentary as seems to be assumed; Fourier analysis (page 61) is left rather as a mystery, when a few pictures could have brought the mathematics to life.

But even with these faults corrected, I should still have doubts, and not only those of a partisan of the solid state who wonders whether it is still so obviously the overriding goal of physics to press on single-mindedly in the pursuit of the fundamental laws. It is not just this, but a feeling (of which some of the specific points just cited are evidence) that the book does not encourage the student to grow as he learns. There are some comparatively elementary texts (Born's *Atomic Physics* springs to mind) to which one can turn again and again, after a spell of more advanced work, to find that the heart of the matter is there; the learner's difficulties are appreciated and overcome without condescension. Such books the serious student will cherish all his life, but this I fear is not one of them. If he comes back to it he will feel that he was talked down to just a little, that the book explained to him little more than what it set out to explain, without any of the shafts of illumination that a great master can give—cannot but give—along the road. In short, it has the makings of a sound textbook; it could not be called, in the highest sense, a work of scholarship. And this a pity, for our best students deserve the best we can offer.

A. B. Pippard

Spinning electrons

Electron Spin Resonance: Theory and Applications. By N. M. Atherton. Pp. x +438. (Ellis Horwood, distributed by Wiley: Chichester; Halsted (Wiley): New York, November 1973.) £11.50.

The theory and techniques of electron spin resonance have now been developed for almost thirty years, and yet the subject still has its growth points, and is still attracting large numbers of research students. The author of this text has seen a real need to provide a basic reference text for new (and existing) workers in this field. Most books on this subject require frequent recourse to other sources, but in this case most aspects of the theory are developed from a sufficiently elementary level to make the text particularly useful to postgraduate physicists and chemists. In that it deals essentially with the theoretical background to the subject, the text forms a useful companion to other recent publications on experimental techniques cited in the bibliography.

The first two chapters of the book deal essentially with introductory aspects of the subject, and form a sound basis from which the subject can be developed. They are followed by two chapters dealing principally with most aspects of the ESR spectroscopy of radicals in solution and trapped in solids. Chapters 6 and 8 deal with the theory of transition metal complexes and with the basic theory of the g-tensor and are followed by two comprehensive chapters on relaxation and lineshape theories. The interpretations of experimental parameters such as g values and hyperfine splittings for simple systems are developed extensively. The sections concerned primarily with transition metal ions tend to stand out in contrast to other sections in that the theory is not developed from quite the same basic level as in the earlier chapters: an inevitable consequence when trying to cover such an extensive subject in so short a space. The problem is helped however, by an excellent bibliography. The final chapters are concerned with two particular aspects of the subject: double resonance and gas phase electron resonance.

The whole range of material covered by the text relies considerably on a basic understanding of quantum mechanics. Though the level of knowledge required is beyond many undergraduate physics and chemistry courses it would require very little effort for most postgraduate students to achieve the necessary standard. The text might have benefited from a brief appendix outlining the principal techniques required such as elementary matrix methods, perturbation theory and some aspects of angular momentum theory. The majority of the topics are developed from an elementary level but unfortunately, although perhaps inevitably, the discussion ceases before many of the interesting examples. Many of the topics treated could have benefited from a few more illustrative numerical examples.

In conclusion I should say that the text forms a very worthwhile contribution which will certainly be a standard requirement for most laboratories working in this field. Many workers in electron resonance spectroscopy have long felt the need for a work of this kind, and I hope that the publishers will consider producing a cheaper paperback edition. The author has undoubtedly achieved his aim described in the preface, namely, to produce a book which will prepare people to read the electron spin resonance literature and that would be useful for a number of years.

E. F. Slade

BIOLOGICAL SCIENCES

Promoting plants

Phytochemistry: the Process and Products of Photosynthesis. Edited by L. P. Miller. Vol. 1, pp. xiv+410; vol. 2, pp. xiv+445. (Van Nostrand Reinhold: New York and London, June 1973.) Vol. 1, £11.25; vol. 2, £12.25.
The Biochemistry of Green Plants. By D. W. Krogmann. Pp. xiv+239. (Prentice-Hall: Englewood Cliffs, October 1973.) £11.95.

PLANT biochemistry has always been a Cinderella subject, and a Cinderella who has not yet met her fairy godmother. Due principally to the attitude of mind that M. D. Kamen has referred to as "mammalian chauvinism", the study of the biochemistry of plant processes has to date been an undeservedly neglected field. Even the rawest biochemistry undergraduate appreciates that the fixation of atmospheric CO_2 by green plants utilising solar energy is the most important chemical process in the biosphere; nevertheless as he pursues his biochemical career, he gradually becomes indoctrinated into the view that those processes which occur in animal cells are of paramount importance and that the study of plant processes is not a suitable field in which to carve a career.

Fortunately, there are signs that this trend is due to be reversed. The consuming requirement for increased global food supplies is bound ultimately to direct attention towards the mechanisms of plant growth and productivity, which surely can only be properly understood at the biochemical level. Both of the books under review attempt in their own completely different way to act as fairy godmothers for plant biochemistry, one by selecting for detailed treatment the currently most fashionable topics within the subject, and the other by dealing exhaustively with the peculiar ability of plants to synthesise substances of fascination for the organic chemist.

In his selective textbook Dr D. W. Krogmann has attempted to show, in terms that an undergraduate can assimilate, the excitement that investigating plant biochemistry can bring. It does not attempt to deal with secondary product biosynthesis, at which plants are adept, but does give intensive coverage of a range of processes which either only occur in plants, or are partiularly well illustrated by plants. These include hexose breakdown, nitrate and sulphate reduction, photosynthesis, cell wall biochemistry, fat metabolism, chloroplast development, photomorphogenesis and hormone action. It is undeniable that there is a substantial gap in the literature which this book attempts to plug. The treatment, however, is rather patchy. The detailed coverage of photosynthesis, hexose metabolism, cell wall synthesis and lipid metabolism is very complete and would be of great assistance to students of plant biochemistry. On the other hand, the book falls down a little in the area of control of metabolism by light and hormones—these, however, are immense subjects in their own right and perhaps the superficial coverage presented here would be considered sufficient by most undergraduates. In any case, the book is a welcome addition to the available literature, not least because it is written in an entertainingly informal style (including verses from Ogden Nash) and yet fearlessly challenges the view that the only really interesting biochemical processes go on in *Escherischia coli* and *H. sapiens* var. HeLa.

The opposite approach is taken by Dr L. P. Miller who has undertaken to edit a three-volume treatise (only the first two volumes of which are yet available for review) on phytochemistry. In contrast with Dr Krogmann's book, the treatise is substance-oriented rather than process-oriented. This will undoubtedly ensure its popularity with organic and natural product chemists,—but will probably mitigate against its purchase by genuine plant biochemists of which in any case there are very few. The first volume deals with photosynthesis, photosynthetic pigments and photosynthetic products, namely the various forms of carbohydrates. The second volume covers the various organic metabolites synthesised by plants including amino acids, proteins, purines and pyrimidines, alkaloids, steroids and flavonoids. The standard of coverage, both in comprehensiveness and depth of detail is consistently of the highest order, as would be expected of the very distinguished contributors to both volumes.

The treatise is clearly not intended as an undergraduate text, however, and it is likely to appeal principally to the research worker and university teacher with specialised interests in natural products. The very scale of the production emphasises the enormous amount of purely chemical information that is available on the substances present in green plants and this treatise should help to accelerate the exploitation of plants for the synthesis of rare and valuable chemotherapeutic agents. As a source book, this treatise should be extremely valuable on the shelves of any biochemical library. Neither of these books will, on their own, pull plant biochemistry out of the doldrums, but they both serve admirably to emphasise the unique and critically important role of plants in the living world. They should be required reading in all biology and biochemistry departments.

H. SMITH

Lithium therapy

Lithium: Its Role in Psychiatric Research and Treatment. Edited by Samuel Gershon and Baron Shopsin. Pp. xi+358. (Plenum: New York and London, 1973.) $22.50.

THE use of lithium for the treatment and prevention of recurrent mental illness has been the subject of a remarkably vigorous debate. This book presents an intriguing mixture of chapters from clinical psychiatrists using lithium therapy and also from various groups who are pursuing some of the many possible lines of enquiry into the mechanism whereby a simple metal ion can restore abnormal mood to normal and actually prevent future severe mood changes. It would perhaps have been interesting if one chapter in the book had been written by a representative of the English group of psychiatrists who have so forcibly questioned some of the claims made for lithium therapy.

The careful and detailed research by Danish workers is clearly explained by

Proboscis



ADULT female Günther's dik-dik, from *The Ruminant Stomach: Stomach Structure and Feeding Habits of East African Game Ruminants* by R. R. Hofmann. (East African Monographs in Biology vol. 2.) (East African Literature Bureau: Nairobi, 1973.) Boards £10.60; paper £6.50.

Dr Mogen Schou in chapters 10 and 14. Dr Schou of course, has done much to promote this treatment by his patient advocacy of the real and unique clinical benefits of lithium therapy in the affective disorders. I particularly liked Dr Schou's suggestions that clinicians should record dosage in millimoles lithium, and that drug suppliers should indicate the lithium content of their preparations in the same units. His plea that research reports should state the timing of blood taken for lithium analysis in relation to the last oral dosage of lithium salt is also worthy of mention.

As a clinical chemist I would have wanted some extension of the remarks in chapter 14, page 292 on the need for proper control of serum lithium analysis—and in this context cannot resist asking for more details of the method which allows Drs Gershon and Shopsin in chapter 7, page 133 to quote serum lithium concentrations to three decimal places. (This in no way detracts from a useful account of lithium toxicity.)

The opening and closing chapters of the book, by Drs N. S. Kline and R. R. Fieve are especially stimulating and outspoken. I was interested, for example, to be reminded by Dr Kline that lithium has a real historical claim to be among the first of the "psychic energisers" and to read that the very cheapness and availability of lithium salts has made drug houses reluctant to market preparations which may compete with other more complex (and profitable) drugs.

In due course it can be expected that lithium will be superseded by more effective and less toxic substances once the basis of its action on mood is clearly understood. Until that rather distant time comes this volume will be required reading for all of those who wish to improve the treatment of patients suffering severe mood disturbance, by the use of well controlled lithium therapy.

G. S. Fell

Runaway chromosomes

Chromosomes in Human Cancer. By J. Cervenka and L. Koulischer. Pp. viii + 203. (Thomas: Springfield, Ill., July 1973.) $13.75.

The stated purpose of this book is to "gather all available information on chromosomes in human tumours with the fervent hope that they might be used as a source of understanding of the seemingly disordered chromosomal constitution in human cancer cells and of the few rules which may be derived from these data". The authors have definitely succeeded in the sisyphean task of gathering the information. The book will be a precious source for the quick identification of sources. Is it presumptuous for one who has no clinical qualification to suggest that the fervent hope has not been fulfilled?

The few rules which emerge are commonplaces of the dynamics of cell populations, and indeed of populations in general. These rules derive from first principles of evolutionary genetics (including cytogenetics) and have been experimentally confirmed with unicellular organisms and with cultured somatic cells and transplants of higher organisms including man.

The value of this book does not lie in illuminating the chromosomal mess in tumours. (Incidentally, is enough known about chromosomal variation in normal somatic tissues, especially embryonic?) It lies in the many tables—at least one for each of the major forms of human cancer—which summarises very clearly the published cases in which chromosomal analysis was performed. For this service those engaged in research on tumours, both clinical and experimental, should be grateful to the authors.

G. Pontecorvo

Life is inorganic too

Inorganic Biochemistry. Edited by Gunther L. Eichorn. Vol. 1 pp. xxiv+ 1–608; vol. 2 pp. xi+609–1263, (Elsevier: Amsterdam, London and New York, 1973.) Dfl. 300; $105.30.

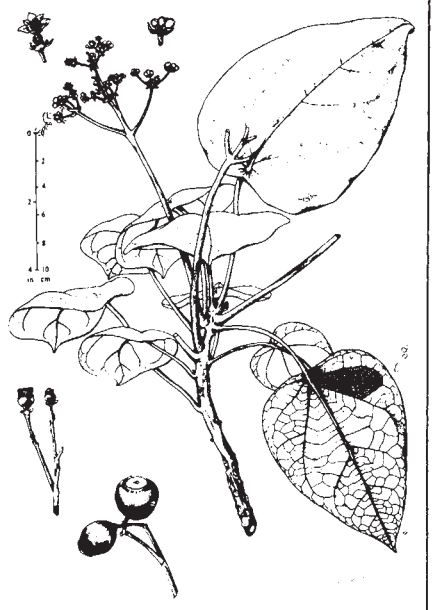
These two volumes do not form a book. Rather they hold together thirty-four booklets running from the necessary inorganic chemistry for the understanding of biological roles of metal ions to the description of the metal-containing biological systems themselves. All the important roles of metals are described but most of the booklets are written by an authority largely for other authorities and in a style which precludes a general reader from getting past the first few pages. Many of the chapters are not therefore readable at a sitting and they could as well have appeared in a review journal where the objective is extensive reference rather than intelligent discussion. In fact it is frequently the case that the biological significance of the materials under discussion is lost among the mass of detail on absorption spectra, EPR signals and Mössbauer data. Striking exceptions are the chapters of Nielands on ion carriers, and by Spiro on phosphate transfer.

I feel that the title of this book should have been 'The Properties of Inorganic Materials found in Biological Systems' for the stress is too far from biological function. The question then arises: is this a book on inorganic biochemistry?

For many years most scientists have looked upon living systems as a growing and self-reproducing organic matrix. Such a view is a natural development from the separation of the animal and vegetable from the mineral kingdoms. The distinction, perpetuated in the educational divisions of inorganic and organic chemistry has forced inorganic chemistry more and more toward physical sciences and organic chemistry toward life sciences. Today one knows that this distinction, seen in almost every chemistry journal and in most chemistry courses in schools and universities is wrong-headed. Life has evolved from inorganic materials (generating organic chemistry as it went) and in that evolution has incorporated every facet of inorganic chemistry which was profitable to it. This book clearly proves this point. The only reason for using a title such as Inorganic Biochemistry is to get away from the implied title 'Organic Biochemistry' every time an inorganic chemist reads 'Biochemistry'. In other words Avis quite rightly wants a share of Hertz's business: car renting, and the study of biology by chemists, is not a monopoly activity. What then is the inorganic part of biology?

The roles of inorganic materials can readily be separated into the functions of bulk structural units, such as shells and bones, control elements, such as energy stores in concentration gradients, and catalyst centres. What feel does this

Tropical tree



Leaves, fruit and flowers of *Hernandia nymphaeifolia*, so called because of the similarity of its leaves to those of the water lily *Nymphaea*. This small tree grows on sea coasts in East Africa, southern Asia and the south Pacific. From *Tree Flora of Malaya* vol. 2, edited by T. C. Whitman for the Forest Department of Malaysia. (Longman: Essex, July 1973.) £12.50.

book give of these different biological topics?

The great structural role of silica, calcium carbonate, and calcium phosphates is not described. The nature of the nerve pulse, of the triggering of muscle contraction, of hormone release, of coagulation, of almost what you will as far as (inorganic) control over action is concerned receives scant mention with one notable exception. The editor has made it his speciality to deal with the interaction of metal cations with the polymers of translation and transcription, RNA and DNA. Yet even in his very interesting chapters he falls into the inorganic trap. He becomes enmeshed in a net of spectroscopic readout. In fact I surmise that the reason this book omits two of the three fields of inorganic biochemistry, solid state structures and control, is that this chemistry belongs to the elements sodium, potassium, magnesium and calcium. The inorganic chemistry of these elements is taught no more because there are no spectra to tease the mind. Out of sight (spectroscopy) out of mind, is true for ionorganic biochemistry and for many other aspects of inorganic chemistry today.

The third function of inorganic elements in biology is catalysis. Catalysis is carried out by transition metal ions or by ions such as zinc which can be replaced by these highly spectroscopic cations. The apparatus of the physical chemist can be tuned in. Each of the very good chapters on iron, cobalt, manganese, copper and zinc systems, all in a variety of forms, shows very well how to examine the active site but, while using these exciting tools, I miss the answers to the questions: Why has biology finished up with a few metals, in a few sites? Why zinc for acid catalysis and not copper? In fact I find myself asking: Who says that the role of these metal ions is as catalysts? Perhaps, through their special stereochemistry, they just generate curious organic chemistry in the active site region.

Is the role of the inorganic component too muddled with the role of the organic to be extractable? Who says ferredoxins are just electron-transfer proteins? I could be persuaded that they are really just electron traps and storage units. Their design is associated with the establishment of an electron at a redox potential close to that of the hydrogen potential but such that they do not blow off hydrogen. If my points are relevant the problem of building inorganic model systems is not what it seems to be for the overriding consideration is a control over thermodynamic not spectroscopic properties and sometimes even a prevention of a reaction. Biology has to store electrons (and elements) and it has to control activity as well as to catalyse reactions. By looking at spectroscopic signals too closely there is the danger that the purpose of biology is lost.

Clearly this book is extremely valuable for the specialist, but when it appears on library shelves (it will not appear anywhere else at $ 105) it must be catalogued under Inorganic and not Organic Chemistry using the real meanings of words.

R. J. P. Williams

Primate morphology

Form and Pattern in Human Evolution: Some Mathematical, Physical, and Engineering Approaches. By Charles Oxnard. Pp. ix+218. (University of Chicago: Chicago and London, 1973.) £5.65.

The scientific basis for primate morphological and evolutionary studies has widened dramatically in the past two decades. This book describes both the more established of these new techniques as well as others whose robustness and application are still being investigated.

Charles Oxnard is the doyen of this rapidly expanding 'technological' approach to primate morphology. It is much to his credit, and to the credit of the schools at Birmingham and Chicago, that hypotheses in studies of both extant and fossil primates are now being tested by experiment and analysis. The examples he gives of the application of these techniques are almost exclusively of work by these two schools and a more cynical reviewer than I might be justified in suggesting that a comprehensive collection of Oxnard's reprints would save the cost of the book!

This somewhat parochial approach has led to two significant omissions. The first is the lack of any assessment of the biomechanical analyses of extant and fossil primate limb material that have been published recently by Preuschoft. Many workers have been waiting for someone of Oxnard's competence in this field to assess the relative proportion of wheat and chaff that is contained in this particular technique.

A second, more important, omission is that allometry is not seriously considered. A significant part of multivariate morphometrics has been concerned with the identification of the effects of size on shape. A review and discussion of the role of factor and principal component analysis in such studies and of the work of Jolicoeur, Gould and Reyment would have been welcome.

Apart from two introductory chapters, and a useful chapter devoted to simple testing procedures, the book is devoted to explanations and the applications of multivariate, group finding, stress and optical data analysis. Without exception these expositions make the most complicated methods plain. The chapters on multivariate morphometrics and optical data analysis are particularly good. It is a shame that in the former chapter, the author has been tempted into making over-zealous phylogenetic interpretations of the results of the shoulder girdle analysis. The new axes generated through the universe of data on the shoulder do indeed seem to have a functional, but not necessarily evolutionary, significance. It would be a pity if this classic study of function and morphology is affected by the fallacy that the proximity and sequence of extant groups is a guide to their phylogenetic relationships. This same fallacy has bedevilled so many molecular biological phylogenetic studies.

A point of minor confusion is the use of factor analysis as an umbrella term to include principal components analysis; there are of course differences between the two techniques.

These criticisms do not seriously detract from any of the books usefulness; I learned much from reading it. Students and research workers would be well advised to buy and read it. Its careful production and accuracy are a credit to the author.

There is a danger that these increasingly complex methods will separate still further the 'experimental' primate morphologists from those who study hominid fossils and evolution. A few people try to do both and it is more important than ever to see to it that further polarisation of these two groups does not take place.

Bernard Wood

Pin down an attitude

Attitudes and Their Measurement. By Nigel Lemon. Pp. vii+294. (Batsford: London; Halsted: New York, December 1973.) £4.20.

There are two schools of thought on the usefulness of the concept of 'attitude' in the social sciences in general and in social psychology in particular. School *A*, to which Dr Lemon belongs, treats 'attitude' very seriously and regards it as "social psychology's central problem", its study being specially important when it is resistant to change and when it clashes with 'social structural' factors. School *B*, to which my sympathies incline, is described by Dr Lemon as "a substantial group who would deny the need for such a term and who would argue that attitude adds nothing to existing explanations of conduct in social structural terms".

The attempt to measure attitude was initiated by L. L. Thurstone in 1929, although as an explanatory device, it had been employed, in 1918, by W. I.

Thomas and F. Znaniecki; and it was pushed into the limelight by G. W. Allport's studies of personality in 1935. The passage of four decades since then, and the hundreds of papers on the subject cited by Dr Lemon, leave the sceptical reader with the suspicion that there is not a great deal to show for all this effort. Dr Lemon does not turn a blind eye to the critics, and he is acutely aware of what he calls "the widening gulf between the increasing sophistication of measurement and the relatively crude conceptualisation on which it is based". He treats attitude as an inference from observations, a latent process, a so-called "intervening variable". It is not, in his view, merely a descriptive expression to be defined as "the probability of occurrence of defined behaviour in a definite situation".

Whatever the explanatory merits of the concept, it must be conceded that Dr Lemon has taken pains with this work. He has immersed himself in the literature, and mastered a wide range of techniques of 'measurement'. He deals with the underlying logic of the subject, with self-administered inventories, with indirect assessment procedures, with the structure of attitudes, and with models of scaling stimuli and responses.

While praising the author's commendable skill in cogent presentation, I nevertheless still wonder how he has succeeded in allaying his own misgivings as to "crude conceptualisation". And the sceptic, whose reservations are not dispelled by a display of technical virtuosity, will continue to seek more convincing explanations of social behaviour.

JOHN COHEN

Enzymes attached

Immobolized Enzymes. By O. R. Zaborsky. Pp. 175. (CRC Press: Cleveland, 1973.) $26.50.

DURING the past decade, many hundreds of scientific papers and tens of reviews, some good, others bad, have been published on immobilised enzymes. Here for the first time is a comprehensive book giving a reasonably balanced and objective survey of the subject.

Much of the published work has been concerned with immobilisation techniques and this is reflected in this book, almost two thirds of which are devoted to chapters describing the various techniques for immobilisation: covalent attachment, intermolecular cross-linking, adsorption, entrapment, microencapsulation and containment by semipermeable membranes. In each of these chapters, many individual methods are given, and the properties of the resulting products are described. The remaining chapters are on enzyme reactors, applications and related topics.

The scientific literature, including patents, is covered in detail up till the end of 1971. With so many scientific publications on immobilised enzymes appearing in the last two years, it is inevitable that certain aspects, for instance treatments of diffusional limitation and reactor operation, are already out of date. Lists of more recent literature are, however, available from the Corning Glass Company, USA. The chapter on applications, both industrial and analytical, inevitably is rather short of data, and as the author points out is not a true reflection of the work that has been done in industrial laboratories which in some cases is far ahead of anything published.

Nevertheless, this is an excellent book and is recommended as a useful introduction and guide for anyone wishing to acquaint himself with immobilised enzymes.

M. D. LILLY

Blue-green biology

The Biology of Blue-Green Algae. Edited by N. G. Carr and B. A. Whitton. Pp. x+676. (Blackwell Scientific: London; University of California: Berkely, 1973.) £13.50.

The Blue-Green Algae. By G. E. Fogg, W. D. P. Stewart, P. Fay and A. E. Walsby. Pp. ii+459. (Academic. London and New York, November 1973.) £8.50; $24.

IN times past it was usual to seek specialised information about the 'biology' of a group of organisms from ancillary sections in a major work on taxonomy, structure and reproduction. The algae were no exception, but the growing number and specialisation of biological disciplines has led to greater dependence upon reviews and occasional multi-author monographs or compendia with emphasis upon physiology, biochemistry, ecology, genetics and fine structure. The first book is a good example of the latter: with 26 contributors it has a pleasantly international flavour. In this respect, and in size and coverage, it differs from the only previously comparable work, edited by Federov and Telitchenko and published (1964) in Russian under the same title.

The 25 chapters, and 4 appendices (on cultures), are often written with considerable individuality and personal outlook—the fascinating contribution on ecology in hot springs by Castenholz is a notable example. As the editors admit, the coverage of the entire subject is uneven; some important fields (such as mineral nutrition, kinetics of growth and photosynthesis) receive only passing mention, although some specialised topics are treated in detail. The single bibliography and subject plus organism indices are valuable features, but the book is basically a collection of independent, authoritative reviews. It constitutes an excellent and readable source of information on very many topics of the relevant biology: for a really integrated account, the reader must turn elsewhere.

He could not do better than to read the second book, *The Blue-green Algae*. Although written by four authors, their contributions merge into an organised pattern of topics and coherent style wherein preferences of individuals can only be recognised by the *cognoscenti*. The brief introduction should stimulate, after which a course is steered through three main phases of structure, physiology and ecology. An early chapter on general form and structure will be the more familiar classical area, and an essential foundation, after which deeper and more recently charted waters are traversed. First appears fine structure, and then aspects of cell physiology. Overall physiology is expressed in a chapter on culture, nutrition and growth, after which follow aspects of metabolism (photosynthesis and chemosynthesis, heterotrophy and respiration and nitrogen metabolism). Structural aspects again come to the fore briefly in an account of reproduction and cellular differentiation, but the rest of the book deals extensively with external relations—in ecology (freshwater, marine and terrestrial), pathogens and symbiotic associations. The exposition is clear and authoritative, the illustrations and figures numerous and helpful, the bibliography excellent for both classic and modern work. In the book's 'connective tissue', linking intensively studied 'nodes' of structure, physiology and ecology, speculation is frequent and enticing—the discussions of vertical stratification in ecology, of plastid origin, and of early evolution provide examples.

The two books are complementary as well as overlapping: though the second is a balanced unity, the first contains more detailed accounts of some topics. The coverage achieved will be the envy of addicts to other groups of algae, and the remarkable array of biological characteristics presented will surprise many. To both works is the comment (on the first) of T. D. Brock apt—"the blue-green algae finally come into their own".

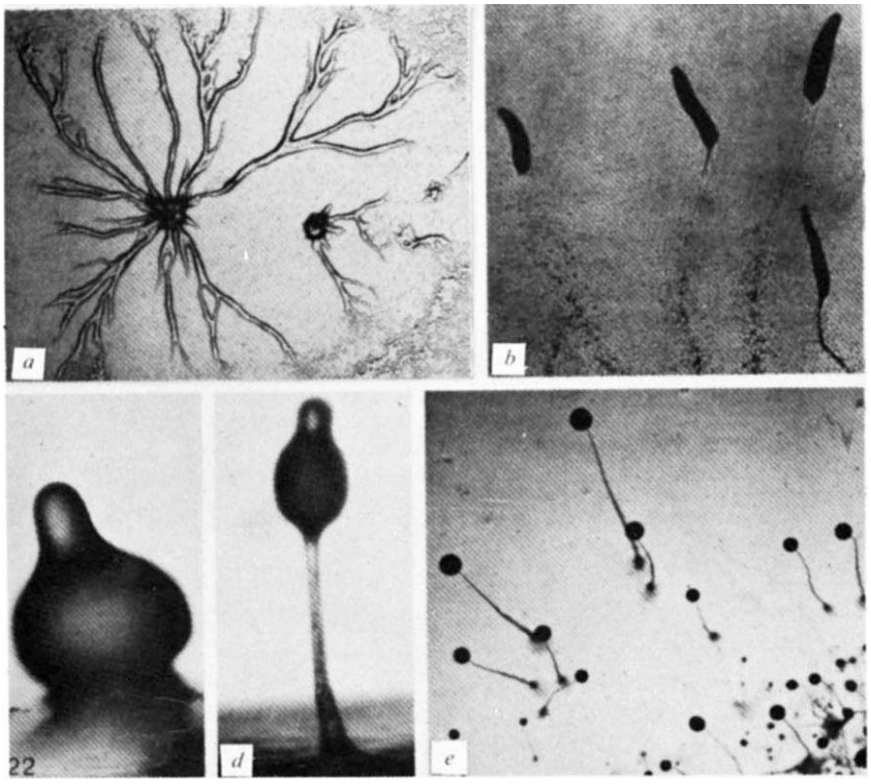
J. F. TALLING

Bigger means better

Evolution of the Brain and Intelligence. By Harry J. Jerison. Pp. xiv+482. (Academic: New York and London, December 1973.) $25; £11.75.

THIS book covers the evolutionary development of the vertebrate brain. The author's views are expressed very clearly and the whole is well illustrated. A large number of tables and graphs gather to-

Slime mould



STAGES in the differentiation of the cellular slime mould, *Dictyostelium discoideum*, from independent myxamoebae to a multicellular organism containing two cell types. *a*, Aggregating myxamoebae; *b*, migrating 'slugs' containing several thousand cells; *c–e*, differentiation of the slug into two cell types, spore and stalk. From *The Fungi: An Advanced Treatise* (vol. 4B, *A Taxonomic Review with Keys: Basidiomycetes and Lower Fungi*), edited by G. C. Ainsworth, Frederick K. Sparrow and Alfred S. Sussman (Academic; New York and London, December 1973.) $28.00; £13.15.

gether much of the original evidence and there is a critical handling of the data and its statistical analysis. Since the evidence presented is mainly palaeontological the size of the brain and its outer shape are the main data. From these data are woven a theory of the evolution of intelligence. As the author rightly says "The most controversial facts in the book will probably be associated with the extensive use of the evidence of relative brain size. There is no debate about the validity of the measure. There is a debate, however, about their meaningfulness".

A trivial answer to the meaning of brain size is that it is an index of the number of nerve cells. The evidence for this is presented. It is not of course possible to discuss the degree or nature of interconnection between the nerve cells by using whole brain size. Since this measure cannot be used, the author accepts the conclusion that bigger means better or at least more intelligent.

But before one can proceed to examine which brains are bigger and therefore better, one must first eliminate what might be called the brain/brawn problem because the most obvious factor varying the size of the brain of animals is the size of the animal itself. This effect is repeatedly and convincingly illustrated. There is a straight-line relationship with a 2/3 slope between the log of the brain weight and the log of the body weight. This applies within a species and between related species. It even applies over pretty widely scattered species. Fish and reptiles make up one line. Birds and mammals including primates make another parallel line with a bit more brain for each ton of body.

This itself has always seemed to me one of the most surprising facts about brains. Why does a little man have a little brain? Why does it take a huge brain to run a huge dog like a Great Dane whereas a much smaller brain can make a fox terrier or a cairn behave just like a dog. There is no evidence that the Great Dane has more data to analyse in proportion to its body mass except for the relatively small input from his muscles. There is no reason why the motor machinery should need to be expanded as the mass of muscle increases.

In spite of this being the basic fact, it is taken by the author as his base line and he really examines deviations from the base line without really attempting to explain the curious fundamental relationship between brain and body weight. Fifty million years ago, early horse brains expanded in relation to their body weight but in subsequent horse development the brain increased only in proportion to the body. That early spurt is used to explain why horses are so smart and the later expansion with body size is accepted as a fact of brain life. By implication, the base line represents "the brain tissue needed to transmit impulses to and from the integrative centres" to quote the author's quotation of Lashley. The excess brain is a measure of behavioural capacity in the author's view.

The facts about the growth of brain size are well presented; the meaning of the data is more puzzling. If the excess of brain size beyond that expected for body size were a measure of behavioural capacity, we should be able to spot our academic and political leaders at a glance and they would in fact be the giants they consider themselves to be.

P. D. WALL

Peat

Peatlands. By P. D. Moore and D. J. Bellamy. Pp. vi +221. (Elek: London, January 1974.) £4.

THE growing interest in peat and in peat-forming ecosystems, both from the ecological and from the commercial points of view, has led to a series of major international peat congresses in recent years. This book, by two of Britain's most knowledgeable and widely-travelled 'peatniks', summarises much of the work that has resulted from these congresses, and provides an invaluable source book on peatland ecology, utilisation, and conservation. In the opening chapters a detailed, and largely original, account is given of the morphologically distinct peatland types recognisable in Europe and North America, and of the climatic, hydrological, and geochemical factors governing their distribution. Of particular interest is the section dealing with the striking surface patterns of peat ridges and open water pools characteristic of some of these peatlands, and with the possible mechanisms by which this patterning arises.

Chapter 4 deals with the processes governing rates of peat accumulation, and with the retention and cycling of mineral ions. Successive chapters then consider structural and physiological adaptations in mire organisms, and the information about peatland development that can be gained from a study of plant remains preserved in the peat. The final chapters discuss the world distribution and exploitation of peatlands, and present a scientific case for more concerted and intensive conservation of peat-forming ecosystems.

Although this book contains much

that is excellent, there is also much that is sub-standard. Surely it was not necessary for the publishers to reduce some of the diagrams to such a size that details are no longer decipherable, nor to split Figs 2.4 and 6.6 each into two halves on non-opposing pages. Misspellings and inconsistencies of usage are frequent, and an almost total lack of hyphens in the text leads to many perplexities: is not the concept of "peat forming vegetation" botanically and grammatically irreconcilable?

This is, then, a book and reference source to recommend to all those interested in peat and peatlands; but one that, because of the abundance of apparently unnecessary ecological jargon in some chapters, will not perhaps be readily comprehended by persons without specialist background knowledge.

JOHN H. TALLIS

Monument to microbes

Microbiology, including Immunology and Molecular Genetics. By Bernard D. Davis, Renato Dulbecco, Herman N. Eisen, Harold S. Ginsberg, W. Barry Wood, jun. and Maclyn McCarty, (Second Edition). Pp. xv+1562. (Harper and Row: Hagerstown and London, 1973.) $27.50.

THIS is an impressive book in scope, size and authorship. Clearly Gray's *Anatomy* can no longer claim a monopoly that, by bulk and weight, it can simultaneously generate a hernia and profound depression in the medical student who handles it! Any student depression initially engendered by the obvious mass of knowledge purveyed in this volume must surely be offset by the subsequent discovery of a pleasant, readable style, clear, helpful diagrams and illustrations, and a splendid layout.

The vital statistics of this second edition command respect—94 of the 1,562 pages are needed to index its 64 chapters! Substantial revision has occurred and new chapters have been introduced to keep pace with the relentless march of knowledge. The untimely death of Barry Wood necessitated contributions on the pathogenic bacteria from a panel of experts (who reveal their presence by eschewing SI units) under the special editorship of Maclyn McCarty. The book is divided into five principal sections comprising bacterial physiology, bacterial and molecular genetics, immunology, bacterial and mycotic infections, and virology, and, as might be expected from such distinguished authors, the overall treatment is very good, with emphasis on scientific method. The avowed intention to produce a text that tells the medical student not only what is known but how it came to be known, has been amply realised.

Inevitably in a book of this size there are points to criticise. Thus the claim that bacterial glycogen synthesis involves uridine diphosphate glucose, confusion between free and standard free energy changes, and omission of the unifying concept of adenylate energy charge when discussing metabolic regulation, are some of the blemishes I noticed. But they do not detract significantly from a monumental work which should provide not only students of medicine and the paramedical sciences but also their biological colleagues with a very useful and comprehensive text.

E. A. DAWES

Deep sea fish

Fishes of the Western North Atlantic, Part 6. (Memoir of the Sear Foundation for Marine Research, No. 1.) Pp. xix+698. (Sears Foundation for Marine Research, Yale University: New Haven, 1973.) $27.50.

THE series of publications under the general title of *Fishes of the Western North Atlantic* is too well known to need introduction. The first volume, produced in 1948, was written entirely by the late H. B. Bigelow and W. C. Schroeder and quickly became a classic source of information on the sharks with which it dealt. It set a standard of excellence which seemed to be almost too high to be maintained in succeeding volumes. Twenty-five years later the sixth part of the series has been published and shows convincingly that the standard is in no way diminished.

Two important policy changes are announced with this volume. First, that in future material will be published as it becomes available rather than in systematic order (and thus waiting for laggard contributors). The second change is to permit publication of new taxa within the volume rather than insisting on prior publication elsewhere. Both changes make good sense as attempts to speed the production of further volumes towards the eventual coverage of the western North Atlantic fish fauna.

Part 6 of the FWNA contains the following contributions: the order Heteromi (Notacanthiformes) by S. McDowell, the suborder Cyprinodontoidei (the brackish and salt-water killifishes) by Donn E. Rosen, the order Berycomorphi (Beryciformes) by Loren P. Woods and Pearl M. Sonoda, the order Xenoberyces (Stephanoberyciformes) by A. W. Ebeling and W. H. Weed, and the Macrouridae (rat-tails or grenadiers) by N. B. Marshall and Tomio Iwamoto, preceded by a general account of the order Anacanthini (the Gadiformes or the cod fishes). To produce a collection of contributions by so many authors is a major feat on the part of the editor Daniel M. Cohen and his editorial board.

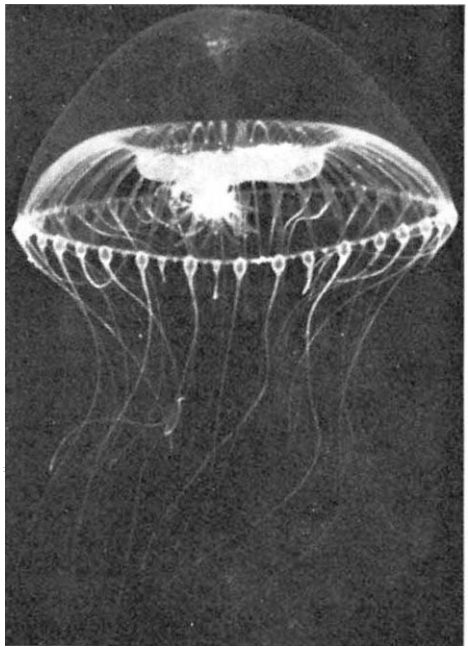
Most of the groups treated in this volume are deep-water fishes which have presented many taxonomic problems in the past. Many of them are also known from relatively few specimens. The value of the volume is therefore very considerable.

McDowell's contribution in unravelling the complicated synonymy of species of spiny eels and halosaurs is a major feat of taxonomy, to which is added a penetrating account of the anatomy and relationships of these fishes. McDowell has summarised the literature and much unpublished information on the distribution and biology, as well as giving detailed accounts of structure and morphology of each species.

Rosen's discussion of the killifishes or euryhaline toothcarps is necessarily different from the other contributions, for this group is primarily freshwater with relatively few euryhaline species. All the marine or brackish toothcarps found on the American Atlantic coast are listed, with notes on their range, clear illustrations, and with a key for their identification.

The berycoid fishes comprise a mixture of deepwater "soldiers", *Hoplostethus* and other genera, together with

Jellyfish



Aequorea aequorea, the largest common jellyfish in the area covered by *Seashore Life of Puget Sound, the Strait of Georgia, and the San Juan Archipelago*, by Eugene N. Kozloff. (University of Washington: Seattle, November 1973.) Boards $15.00; paper $6.95.

the squirrel fishes (Holocentridae), mainly shallow water tropical fishes. Again, many taxonomic problems were evident in this group which Woods and Sonoda have clarified. It is comforting to see such a troublesome spectre as *Hoplostethus islandicus* of German fishery workers finally laid as a junior synonym of *H. atlanticus!*

The contributions of Marshall and Iwamoto have brought together the widespread literature on the rat-tails (Macrouridae) in a way which will facilitate the identification of these difficult fishes. An elucidation of this group is particularly timely as the rat-tails, the dominant fish group of the lower continental shelf, are becoming increasingly attractive as commercial fishes. Russian exploitation of the northern *Coryphaenoides rupestris* has already reached considerable proportions and wider interest in this relatively well-flavoured fish can be predicted.

ALWYNE WHEELER

Taxonomy without genes

Numerical Taxonomy. The Principles and Practice of Numerical Classification. By Peter H. A. Sneath and Robert R. Sokal. Pp. xv+573. (A series of Books in Biology.) (Freeman: San Francisco, October 1973.) £9.40.

THIS is not a second edition of Sokal and Sneath's well known 1963 book on numerical taxonomy but a completely new work. The authors' aim is to provide a comprehensive and up to date account of the subject and to illustrate the advantages of numerical over conventional taxonomy.

It is certainly a very careful and detailed review, and will be of great value to anyone with a professional interest in this field. It is not a book for someone wishing to 'find out about numerical taxonomy'. Readers are referred to the original papers for many of the details and the mathematical notation, although logical and consistent, produces such mind-bending conjunctions as

$$t_{J,1i} = \sum_{i\,J}^{i\,J} X_{ijJ}.$$

When these ingredients are added to the already gross terminology of conventional taxonomy the result is hard going even for people experienced in the field.

The first three chapters on taxonomic principles and evidence provide the background for the rest of the book. The choice and coding of characters is discussed and the authors make it clear that this is the least satisfactory aspect of numerical taxonomy. Chapters 4 and 5 on taxonomic resemblance and structure form the core of the book and chapter 6 deals with methods of estimating cladistic relationships. This last should be particularly useful as it collects together, for the first time, a lot of recently published material. There is a chapter on identification, including the automatic generation of keys; a chapter on populations; a chapter on nomenclature; and a few bits and pieces. The bibliography is vast and there is also a list of applications of numerical taxonomy to systematics classified by the group of organisms. An author and subject index ends the book.

The authors' basic position (highly paraphrased) is that a purely phenetic classification system would have great advantages for biology in general. Genetic and phylogenetic information should be irrelevant to the classification (however interesting in its own right). Old ideas about phenetic resemblance estimating genetic resemblance have been thrown overboard and the good ship SS Phenetics is now sailing under its true colours. The authors accept that the lack of a satisfactory definition of 'character' is a theoretical weakness, but claim a certain robustness in practice. They also state that, in time, numerical taxonomy will become objective, and criteria for grouping will be agreed by common consent.

The very size and complexity of the book makes this last claim seem unlikely. A tremendous variety of techniques is mentioned and each new alternative to some act which was previously without alternative doubles the number. It may be that a book such as this is an essential first step—an inventory before taking the vote; but it seems to me more likely that what will emerge from numerical taxonomy is a set of very useful mathematical methods but no general agreement about the way in which the results of applying them should be used.

Whether one is sympathetic to the aims of numerical taxonomy or not it must be granted that to have written a book as up to date and comprehensive as this is a remarkable achievement. It will be an indispensible reference work for many years to come, proving useful to all taxonomists, not just those who obey the fiat of pheneticism.

M. HILLS

Mineral metabolism

Calcium and Phosphorus Metabolism. By J. T. Irving. Pp viii+246. (Academic (Harcourt Brace Jovanovich): New York and London, September 1973.) £15.

INTEREST in this book is quickly aroused by its table of contents since almost every aspect of the subject is covered in an orderly and well-tabulated progression. The style is readable and there is no difficulty in locating particular topics of interest. The book is reasonably elementary, its depth being necessarily limited by its size, but so much recent knowledge has accumulated, for example with respect to the chemistry of parathyroid hormone, to cyclic AMP and to vitamin D metabolism, that a simple overview such as this is timely. The author might perhaps have made still more use of the opportunities for critical judgment that a monograph provides but attempts to reconcile conflicting evidence are nevertheless made in a rational way. While the overall approach of the book is physiological, biochemical and clinical aspects are interwoven.

The revision for this edition is a little uneven and a few sections are still composed from largely primitive sources. Occasional statements are misleading such as the bald comment that "in myxoedema the level (of blood calcium) is unchanged". The disadvantage of long publication time is apparent particularly in the expanding field of vitamin D metabolism where the most recent references are over 2 years old. Nevertheless this remains an excellent, concise book for students of the field and will also be of value to specialists when they require to look into other physiological aspects of their subject.

T. C. B. STAMP

Ukrainian hunters

Ice-Age Hunters of the Ukraine: Prehistoric Archaeology and Ecology. By Richard Klein. Pp. xviii+140. (University of Chicago: Chicago and London, 1973.) £2.95 boards; £1.35 paper.

THIS small book serves two purposes. First it is a very welcome up to date summary of Soviet research in the upper Palaeolithic of the Ukraine, and, second, it makes available material not readily accessible to Western readers who do not read Russian fluently.

The author takes the occupation of the Ukraine during the Last Glaciation, roughly from about 45,000 to 10,000 years, which covers the Mousterian as well as the Upper Palaeolithic.

The preface and the early sections suggest that the author has undergraduates in mind and this has had some effect on the whole tone of the book, but this approach in no way detracts from its usefulness. Both the archaeology and the supporting studies, geology, fauna and pollen studies, are dealt with in some detail with the result that the area and period are dealt with as a whole, including discussion of the environments during the three stages of the Last Glaciation. This evidence is illustrated by maps, pollen diagrams and faunal lists.

The archaeological side, which is the main purpose of the book, deals very fully with the evidence for dwelling

structures, the earliest of which date from the Mousterian. These houses are mostly constructed from mammoth bone, an animal which forms the bulk of the economy, and whose bones (skulls, long-bones, scapulae and tusks) in a region with a timber shortage, made good building material. In addition to hut plans and their reconstruction, the author reproduces some of the archaeological material, but unfortunately, though being quite generous with his illustrations of the bone objects, gives no useful illustrations of the flint assemblages.

When one looks at the list of references at the back one can only be very grateful for the mass of material that the author has condensed. This is a very useful book and most prehistorians, not excluding Russians, will be glad of it.

J. D'A. WAECHTER

Statistics for biologists

Biostatistical Analysis. By Jerrold H. Zar. Pp. xiv+620. (Prentice-Hall Biological Sciences Series.) (Prentice-Hall: Englewood Cliffs, NJ, 1974.) $25.95.

WHETHER or not statisticians regard the practice as desirable, many biologists choose to be responsible for their own statistical analyses. To aid them, they have a great many bad and a few good cookery books. Professor Zar's thorough presentation of detailed formulae and abundance of worked examples places him firmly in the second category. Those who are prepared to handle a rather heavily formalised symbolism should welcome this well-produced text. The descriptions of analysis of variance calculations and their associated tests of significance are particularly clear. Perhaps the chief weaknesses are the lack of critical guidance on choice between methods (when should a non-parametric technique be adopted for analysis of a continuous variate?) and the unfortunate habit of presenting the scaffolding of tests of significance without summary tables of means.

More than 200 pages of tables of statistical functions are included. Today's computer facilities, and the availability of keys for standard functions on relatively cheap desk calculators, could have allowed substantial omissions and condensations here, with consequent reduction in the high cost of the book. The attitude to computing, indeed, is surprisingly old-fashioned. Little is said about any sophisticated programming. Such uses of computers as are described are concerned merely with performing the heavy arithmetic and with the barbarity of floating point output.

The book evidently originated in a concern for statistical analysis of observational data rather than planned experiments. This emphasis is valuable, though it could usefully have been supported by more discussion of sampling of biological populations and materials, selection of observations and measurement procedures. Particularly interesting is the chapter on distributions of angular directions, a topic rarely mentioned in elementary texts.

D. J. FINNEY

Wobble in the glance

Eye-Movements and Visual Perception. by R. W. Ditchburn. Pp. xv+421. (Clarendon: Oxford; Oxford University: London, December 1973.) £9.50.

WHEN we wish to scrutinise an object and look at it in great detail we point our eyes towards the thing and hold them quite stationary—or so one might think. But even during periods of concentrated fixation, the eyes are never really still. They move constantly with three components of motion: a slight tremor (about 50 Hz, amplitude about 20 arc s) probably originating in the intrinsic noise of the extraocular muscles; slow drift (velocity up to 20 arc min s^{-1}); and intermittent, tiny, fast flicks (usually one or two per second, mean amplitude 5 arc min).

Our normal persistent perception of the visual world must depend on one or more of these apparently involuntary movements because if an optical device is used to hold an image more or less fixed on the retina, despite eye movements, the pattern seems to fade virtually completely within a few seconds. Isaac Newton was perhaps the first to recognise this necessity for a moving retinal stimulus. He noticed that pressing the side of the eyeball with a finger stimulates the retina mechanically, producing a coloured blob superimposed on the visual field; however "if the eye and the finger remain quiet these colours vanish in a second minute of time, but if the finger be moved with a quavering motion they appear again"[1].

Since Newton's time there has been more than a little research on the small eye movements of fixation and the curious phenomena that occur when the retinal image is stabilised. Ditchburn's book is a critical catalogue of this work, invaluable to the researcher in this area and of interest to every student of vision. This book, then, has a place as a companion (but not a complete successor) to the highly personal account of eye movements and visual perception by A. L. Yarbus[2].

Ditchburn begins with a general introduction to visual optics and the problems introduced by movements of the eye. He then provides a most useful review of all the methods of recording eye movements and evaluates them critically. This section, together with an appendix containing many technical details, is of great value to anyone intending to work in this field where instrumentation is so varied in scope and quality and where the recording apparatus itself imposes severe restrictions on the kinds of movements that can be accurately monitored.

Next comes a survey of the results of these recording experiments with a wealth of quantitative data from many different workers. Ditchburn then discusses the various kinds of apparatus used for producing a more or less stationary retinal image despite movements of the eye. Most involve the wearing of a closely fitting contact lens with various appendages such as a sucker, to hold the lens tightly to the cornea, a mirror on a long stalk or even a miniature projection system complete with lens and light bulb. Some of these devices would have made the Spanish Inquisition green with envy. Unfortunately not all these methods are equally efficient in holding the retinal image stationary: consequently the various introspective reports on changes in colour, brightness and clarity of stabilised images are not all equally valid and it is sometimes difficult to know how to evaluate them.

Before considering the causes of perceptual fading of a stabilised image and the function and control of small eye movements, Ditchburn reviews briefly the relevant physiology of the visual system. But let me be clear: this is not the place to learn about the latest advances in visual physiology. This is perhaps the weakest section of the book, but is adequate for the question in hand.

Ditchburn concludes that no single type of small eye movement (tremor, drift or saccade) is adequate to maintain the normal visibility of the image. He believes that both drifts and saccades are essential. It is worth mentioning that not everyone agrees. Steinman and his colleagues[3], for instance, have found that some observers can learn to suppress small saccades completely, without decreasing the accuracy of fixation or the visibility of the target.

Finally, a word about the index. The book is organised into numbered subsections of each chapter and these are used (rather than page numbers) in the index. No doubt this is a great economy in editing and layout but it is a very clumsy system to use. I hope that any future edition of this book may have a real index as well as answering many of the unsolved questions in this fascinating field.

COLIN BLAKEMORE

1 Newton, I., *Opticks*, (Bell, London, 1730).
2 Yarbus, A. L., *Eye Movements and Vision*, (Plenum, New York, 1967).
3 Steinman, R. M., Haddad, G. M., and Skavenski, A. A., *Science*, **181**, 810 (1973).

Fragmentation of the Alpine orogenic belt by microplate dispersal

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Reconstruction of the positions of western Mediterranean microplates in the Oligocene leads to recognition of a formerly continuous 'Alpine' (Mesozoic-Eocene) orogenic belt. This Alpine belt was fragmented by Miocene to Quaternary microplate dispersal in which four episodes of movement are recognised.

It is becoming increasingly clear that the movement of microplates such as Corsica-Sardinia[1,2] and Spain[3] has played a principal role in the tectonic evolution of the Mediterranean but it is difficult to find clear evidence for the exact time and manner in which these and other microplates have moved[4,5]. Here we attempt to reconstruct the history of late Tertiary microplate movement in part of the western Mediterranean; the evidence will be reviewed in more detail in a forthcoming paper. The first step in this study is to determine the palaeogeography in the Oligocene, before the latest episode of microplate dispersal; the second step is to determine the pattern of movement of the microplates. If the reconstruction given here is correct, it explains the longstanding question of why the Eocene orogenic belt of the Alps terminates in northeast Corsica and clarifies the origin of the Miocene orogenic belt of Italy, Sicily, and North Africa (the Apennine-Maghrebian fold belt).

Oligocene palaeogeography

Figure 1 shows a reconstruction of western Mediterranean palaeogeography in the Oligocene, that is, after the 'Alpine' orogeny in the Cretaceous to Eocene but before the 'Apennine-Maghrebian' deformations of the Miocene. The first step in making this reconstruction is to place Corsica and Sardinia next to Provence. Evidence for this fit has been reviewed[1]; further evidence from palaeomagnetism[6,7] and geological matching[2,8] has been reported. Corsica and Sardinia are separated by the Straits of Bonifacio, an opening which widens westwards and which may be a 'microsphenochasm'[4,8]. A greater rotation for Sardinia than for Corsica has been assumed here[8] in order to close the Straits of Bonifacio, improve the geometrical fit with France and better satisfy the palaeomagnetic data[7]. The 'eugeosynclinal' sequences occurring as allochthonous nappes and slabs in the northern Apennines were originally deposited in the area between Corsica and Italy[9,10], and the internal (that is, originally south-western) parts of the eugeosyncline must have been within the Alpine belt since they were deformed in the Palaeogene[10–12]. The external parts of the eugeosyncline lack a Palaeogene unconformity and were thus outside the Alpine belt.

As the next step we propose that the crystalline Calabrian 'massif' of southernmost Italy and NE Sicily should be placed next to Sardinia. This massif, 200 km long and 50 km wide, links the NW–SE trending Apennines with the east-west Maghrebian trends. It is a unique element in the otherwise sedimentary sequences of the Apennines, but most of its characteristics are repeated in the two Kabylie massifs of Algeria and the Rif massif of Morocco. The Calabrian massif is split into three segments by the Straits of Messina and the Catanzaro lowland; we suggest that these transverse depressions may be 'microsphenochasms' due to fragmentation of the massif during its south-eastward movement.

Evidence for the former position of Calabria shown in Fig. 1 is provided by (1) similarities with Alpine Corsica; (2) sediment supply requirements; (3) similarities with the Kabylie massifs; (4) the present movement of the massif and (5) displacement of the Numidian flysch-crystalline massif suture 300 km south-eastwards from Tunisia to NE Sicily (discussed in the final section).

(1) Calabria is not, as has often been implied, a Hercynian massif unaffected by tectonism since the end of the Palaeozoic. Rather it is a stack of nappes (some containing Hercynian crystalline rocks) that, according to some authors[13–15], were emplaced during the Eocene, that is, at the same time as the nappe emplacement in NE Corsica[16]. The direction of movement of the Calabrian nappes is currently a matter of debate, so this aspect cannot be compared with Corsica, but there are close analogies in lithology and metamorphism (high P/T) between Calabria and NE Corsica. Both areas contain phyllites and schists with ophiolites and metabasic rocks—the *'schistes lustrés'* of Corsica[17–20] and various units with controversial nomenclature, some of which have been referred to as *'schistes pseudolustrés'*[21], in Calabria[13,22–24]. Blueschist metamorphism is found in both areas[13,24–26]; elsewhere in the western Mediterranean it is known only in the French Alps[27] and in the Betic Cordilleras[28]. All four areas are part of the reconstructed Alpine belt of Fig. 1.

(2) Two sedimentary units on or adjacent to the Calabrian massif (Fig. 2) seem to require sources of detrital material in Sardinia: the Tusa andesitic flysch, recently shown to be late Oligocene-Aquitanian in age[47] seems to be a trench deposit[30,31] and the late Oligocene-early Miocene volcanic province of western Sardinia seems to be the only available source of calc-alkaline detritus[29,30]; the transgressive Capo d'Orlando flysch, which covers the NE Sicilian part of the Calabrian massif and is probably an arc-trench gap deposit[29],

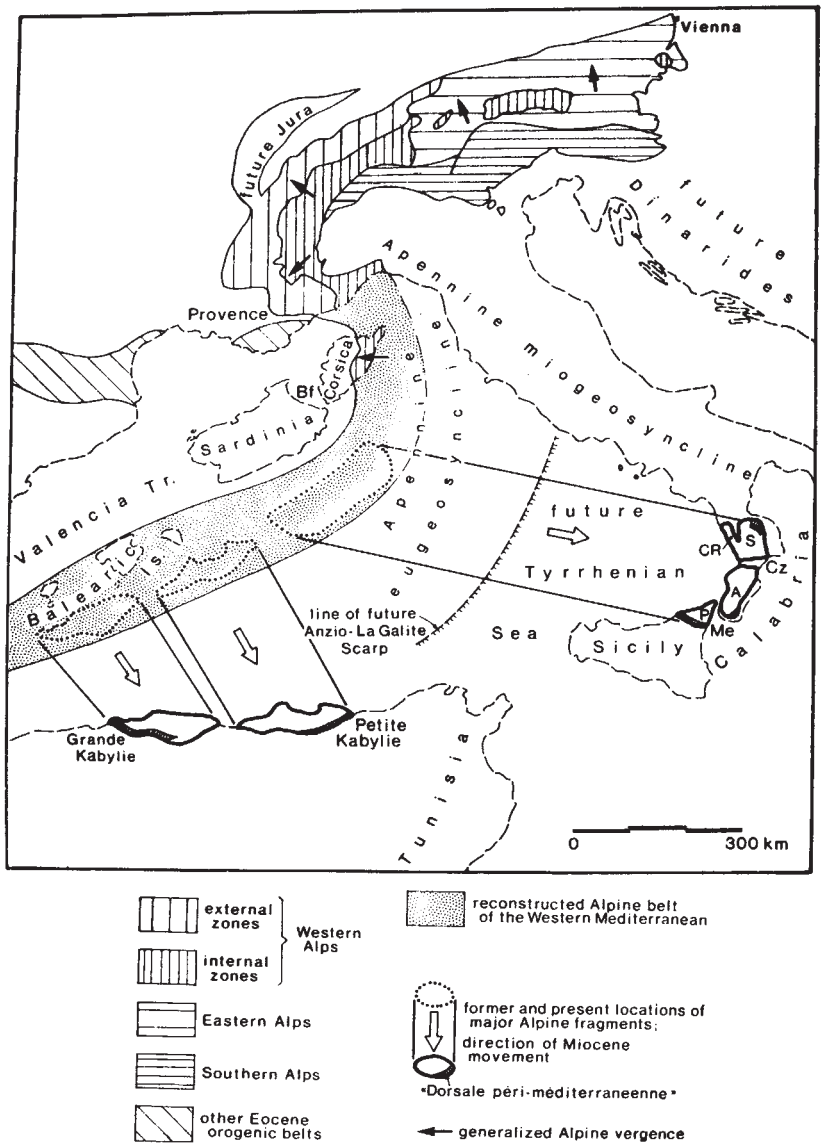


FIG. 1 Reconstruction of the locations of western Mediterranean microplates during the Oligocene after the final Alpine phase in the Eocene but before the Apennine-Maghrebian movements in the Miocene. The probable closure of the Valencia trough has not been shown since data for accurate reconstruction are lacking. This interpretation uses a greater angle of rotation for Sardinia than for Corsica thus closing the probable sphenochasm of the Straits of Bonifacio (Bf). The present positions of the Alpine fragments are shown by heavy lines. The Calabrian 'massif' is broken into three parts by the Catanzaro (Cz) and Messina (Me) sphenochasms. CR, Coastal Range; S, Sila; A, Aspromonte; P, Serra San Bruno; P, Peloritani Mountains.

contains clasts of a red porphyry found in place in Sardinia but not in southern Italy or Sicily[32].

(3) We also note the similarity between the Calabrian and the Kabylie massifs in orogenic timing[33,34] and in the precisely analogous Mesozoic cover of the 'Dorsale péri-méditerranéenne'[35] ('Chaîne calcaire') in the two areas[33,36]. The 600 km gap between these massifs and the northward projection of the Tunisian shelf make either submerged continuity or separation as a result of east–west motion highly unlikely, but the similarities are explained well by the diverging motions shown in Fig. 1.

(4) The active volcanic and seismic zone beneath Calabria reveals ESE-directed motion of the massif[37–40], indicating that Calabria is at present moving in the direction required by the reconstruction of Fig. 1.

A recent report of basement nappe movement of Alpine age in eastern Sardinia[41] suggested that Alpine Calabria might have been located very close to Sardinia. Our field results[42] however, show that the 'basement nappe' is simply a massive landslide of incompetent phyllite off the side of an early Tertiary horst. Thus Sardinia must have been well outside the zone of Alpine compression. We tentatively assume that the elongated seamounts of the Monti di Quirra and the Monti delle Baronie, which trend north–south about 50 km east of Sardinia[43] and from which crystalline rocks have been dredged[44], are part of the Sardinian continental crust. This suggests that the Calabrian block should be placed east of these seamounts, 50-100 km east of the present coast.

Although less evidence is available than for the preceding fit, we make an analogous reconstruction to the southwest, first closing the Valencia trough[45] and then placing the Kabylie massifs against the Balearic Islands on the basis of the Calabria–Kabylie similarities already noted and of the Sardinia–Kabylie similarities discussed by Vardabasso[46].

During the Oligocene and early Miocene there must have been an oceanic basin south-east of Calabria with the Tusa andesitic flysch[47], discussed previously, deposited in a trench on the north-west side, and the continental rise deposits of the Numidian flysch on the south-east side[30]. According to one of us, the Numidian quartz sands were derived from the Saharan shield[48–50]. (Arguments, based on thermoluminescence, for a Sardinian source for the Numidian quartz have recently been shown to be invalid[51].) The distribution of the Numidian shows that the Oligocene African continental margin extended from southern Spain and Morocco through north Africa and Sicily to the Anzio-Ancona line in central Italy[29,49]. Not all this margin however, was necessarily backed by African sialic crust; it has been proposed[4,52] that in the Jurassic an ocean basin opened between Sicily and Italy, so oceanic crust would have been continuous from the site of the present Tyrrhenian Sea into the eastern Mediterranean.

Characteristics of reconstructed Alpine belt

In plate tectonic terms the Alpine orogenic belt is generally considered to be a suture marking the closure of a small

ocean that opened in the Jurassic and early Cretaceous and closed with major episodes of crustal consumption in the middle of the Cretaceous and the Eocene[52]. The Alpine belt as reconstructed in Fig. 1 comprises the Alps proper and NE Corsica, then trends SW through Calabria and the Kabylies, and may continue into the Betic Cordilleras of southern Spain, although the orogenic history of the latter area is not yet well understood[53,54]. From the western Alps as far as the nothern part of the Calabrian massif (Coastal Range, west of the Sila) the Alpine belt is characterised by 'schistes lustrés,' ophiolites, and blueschist metamorphism. From the northern Sila (Longobucco[36,55]) through NE Sicily and the Kabylies to the Rif and the western Betic as far as Granada[35], a new element, the 'Dorsale péri-méditerranéenne' or 'Chaîne calcaire,' is continuously present[36,56]. If this change had occurred between two Alpine fragments, it might have cast doubt on their continuity. But, since western Alps-type characteristics and the 'Dorsale péri-méditerranéene' overlap within a single Alpine fragment and since Eocene orogeny is characteristic of all the fragments, the change does not argue against the proposed reconstruction of the Alpine belt.

The reconstructed Alpine belt of Fig. 1 trends WSW from Vienna to the Kabylies and possibly to Gibraltar, with an offset in the French Alps. Study of magnetic anomalies in the Atlantic[57] has permitted Pitman[52] to determine the successive poles of Africa–Europe rotation. From the early Eocene (Ypresian; anomaly 21, 53 m.y.) to the Tortonian (anomaly 5, 9 m.y.) the average pole was at about 34.4°N, 2.5°W in the European reference frame, or about 300 km southeast of Gibraltar, and Europe and Africa approached each other with a rotation of 6.8°. This rotation probably took place mostly in the Eocene, a time of intense tectonic activity. An Africa–Europe rotation of 6.8° about this pole would cause crustal shortening of 100–200 km in the Alps, which may be compared with geological estimates ranging from 100 to 600 km[58]. Thus the Alpine belt was apparently formed by crustal shortening and/or consumption during the approach of Europe and Africa; this is, of course, the classical explanation of the Alps proper.

The offset in the Western Alps may be due to a projection of the front of the overriding African plate, that is, the projecting Italian block may have pushed the main part of the Alps further to the north. Alternatively, the offset may reflect a trench–trench transform fault following a small circle having its centre at the pole of rotation near Gibraltar. A third possibility is that the Corsica-Sardinia block was part of the Iberian plate while the latter was rotating during the Eocene[3] and that as this plate moved eastward it distorted the Alpine belt and produced the curvature.

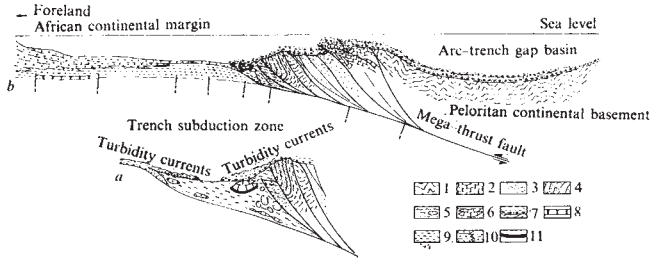


FIG. 2 Front of the rotating Sardinia-Calabria microplate during the early Burdigalian (second episode of movement). This reconstruction is based on geological relationships in NE Sicily and shows the Peloritan basement (Sicilian part of the Calabrian 'massif') overriding oceanic sediments north of the African continental margin prior to collision with Africa in the early Langhian. *a*, Detail of the trench shown in *b*. 1, crystalling basement; 2, limestones of the *'Dorsale péri-méditerranéenne'*, 3–6, flysch and mélanges on the south side of the Calabrian massif; 7, Reitano–Capo d'Orlando flysch (arc–trench gap turbidites); 8, Subsided Panormide carbonate platform; 9, Pre-flysch of the African continental margin; 10, Numidian continental-rise sediments; 11, Radiolarites and diatomites related to volcanic episodes. This cross section is discussed in detail by Wezel[29].

Fragmentation and dispersal of microplates

In France, Switzerland and Austria the Alpine suture was caught between the two large sialic blocks of Europe and Italy. In the western Mediterranean it seems that an oceanic area remained not far southeast of the suture[4,52] and that the microplates moved into this area. The reason for the microplate movement is not clear, although it may be related to buoyancy of crustal material subducted earlier, during the suturing. It has been suggested[59–61] that the movements are due to back-arc extension or 'multispreading' similar to that of the western Pacific[62]. We note, however, a possible difference: while in the western Pacific general subduction of the Pacific plate under Asia produces local upwellings that drive the island arcs back over the Pacific plate, in the Mediterranean there does not seem to have been a general subduction (for example, at present, Calabria alone is moving ESE over the Ionian Sea[37,40]). It seems that the upwelling is the primary driving mechanism, producing only local subduction. Thus in a given episode we expect to find first volcanism, then crustal separation and microplate movement with local subduction.

On the basis of available data four episodes of microplate movement can be tentatively identified in the Tyrrhenian Sea area. The first two episodes probably had analogues to the southwest in the Balearic–Kabylie region; the third and fourth episodes are much more difficult to interpret than the first two.

The first episode involved initial rotation of the Corsica–Sardinia–Calabria microplate away from France and formation of the first part of the Ligurian Sea behind the rotating microplate. The beginning of this episode is marked by the initiation of andesitic volcanism in Sardinia in the late Oligocene—a volcanism which continued until the beginning of the middle Miocene[5]. Graben formation accompanied the volcanism in the Oligocene[63,64] but crustal separation in the Ligurian Sea area need not have occurred immediately. We propose that the separation came in the Aquitanian, since this is the time of a rapid, regional, marine transgression over continental deposits in the Campidano lowland of SW Sardinia, an early Tertiary graben which was ideally situated to record a marine ingression at the beginning of rotation[65,66]. (Analogous opening of the Valencia Trough probably occurred at the same time; this is supported by an age date of 22-19 m.y. on andesitic rocks cored at DSDP site 123 (ref. 45).) The end of the first episode came during the late Aquitanian when Corsica collided with the sialic crust of the northern Apennines, a collision marked by orogenic activity in the eugeosynclinal sequences of the northern Apennines[12,67].

Corsica was then unable to rotate further and the second episode began as the Sardinia–Calabria microplate broke away from Corsica and continued to rotate, forming the rest of the Ligurian Sea and the microsphenochasm of the Straits of Bonifacio. This separation took place in the early Burdigalian, as is shown by marine transgressions of that age in the Bonifacio basin of southern Corsica[68] and in its continuation, the Capo Testa basin of northern Sardinia[69]. Volcanism related to this opening cannot be separated from the rest of the Sardinian Oligo–Miocene volcanism. The palaeomagnetism of volcanic rocks intercalated with fossiliferous marine sediments at Castelsardo in northern Sardinia indicates that Sardinia rotated rather suddenly in the early Miocene[6]; further studies[7] suggest rotation in the late Aquitanian. These dates agree well with the timing proposed here. Figure 2 shows conditions at the leading edge of the Sardinia–Calabria microplate during the second episode of rotation, with the Peloritan continental basement (part of the Calabrian

'massif') approaching the African continental margin. This second episode terminated with the collision between the rotating microplate and the Tunisian margin of North Africa. In this collision the NE Sicilian part of the Calabrian massif (Peloritani Mountains) overrode the Numidian flysch deposits of the African continental slope—orogenic activity that

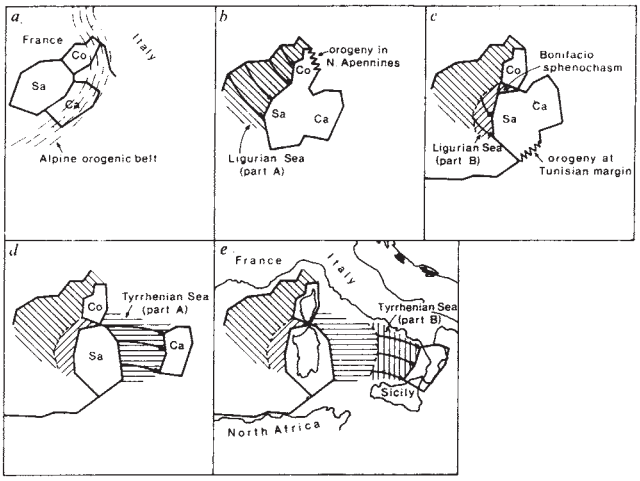


FIG. 3 Schematic diagrams to show the four episodes of tectonic evolution proposed in this paper. *a*, Reconstruction end Oligocene. *b*, After first episode, late Aquitanian. *c*, After second episode, early Langhian. *d*, After third episode, Tortonian. *e*, After fourth episode, present day. Co, Corsica; Sa, Sardinia; Ca, Calabria.

can be dated[29] as early Langhian (upper part of zone N-8 of Blow[70], about 14 m.y.). Collision of the independent but analogous Kabylie microplate(s) with North Africa took place very slightly earlier (lower part of zone N-8 of Blow, about 14.5 m.y., M. Durand-Delga, personal communication, 1973). The line of collision of the Sardinia–Calabria microplate with North Africa is preserved today as the submerged boundary between the Sardinian crystalline block and the Numidian flysch in the Straits of Tunisia[71,72]. Collision of the rotating microplate with the continental mass of North Africa seems to have stopped the rotation of Sardinia, while the Calabrian portion of the microplate, facing the oceanic crust of the eastern Mediterranean-Ionian Sea, broke free and continued to move to the south-east, carrying the line of collision of the Peloritani Mountains with the Numidian about 300 km south-east to its present position.

If the reconstruction of Fig. 1 is correct, the Tyrrhenian Sea was formed in the wake of the Calabrian massif as it moved south-eastwards, but general agreement has not been reached on the time and mode of origin of this sea, and the question of spreading as against oceanisation in place is a matter of continuing debate[45,73,75]. There are conflicting views on the age of the basin. At DSDP site 132 upper Miocene (Messinian) shallow-water evaporites are abruptly overlain in transgression by deep-water Pliocene pelagic ooze ('Trubi'); this led to the interpretation, generalised for the entire Tyrrhenian Sea, that the basin was formed before the Messinian, was dried out by evaporation during the Messinian and was suddenly flooded at the beginning of the Pliocene[45]. On the other hand, a widespread Middle Pliocene transgression analogous to the Middle Pliocene transgression seen on land[75], has been suggested on the basis of dredge hauls and seismic reflection profiles[44], implying that the present basin is Middle Pliocene or younger. We suggest a possible reconciliation of these views by noting that the Tyrrhenian Sea is divided into two parts by a NE–SW trending boundary, here called the 'Anzio–La Galite Scarp'. To the north-west of this scarp is an abyssal plain without volcanoes at about −2,800 m that is underlain by horizon 'M' (top of the Messinian evaporites); DSDP site 132 is in this area. North-south ridges[43,74] emerge northwards from this abyssal plain, apparently forming a continuation of the tectonic ridges of the northern Apennines. To the south-east of the scarp is an abyssal plain at about −3,400 m, marked by large Plio–Pleistocene volcanoes with a NNE–SSW elongation[43,74]. We have not been able to trace the distinctive seismic horizon 'M' across the Anzio–La Galite Scarp into the SE Tyrrhenian, nor to recognise it anywhere in this area. Thus we tentatively conclude that the Messinian evaporites are not present in the SE Tyrrhenian, at least not in the very thick, evaporated, shallow-water deep-basin facies of DSDP site 132. The SE Tyrrhenian basin may thus be post-Messinian. The possible difference in age between the two parts of the Tyrrhenian basin leads us to suggest two further episodes of microplate movement, although these are more speculative than the first two.

After collision with Tunisia the Sardinian part of the microplate was no longer free to move and the third episode began with separation of Calabria from Sardinia. This separation must have been post-Aquitanian (if the Sardinian volcanics were in fact the source of the latest Oligocene-Aquitanian Tusa andesitic flysch on the south-east side of the Calabrian massif[47]), later than early Langhian (assuming that Sardinia and Calabria were attached until Sardinia was blocked by Tunisia) and pre-Messinian (because Messinian evaporites were deposited in the western Tyrrhenian at DSDP site 132 ref. 45). The Miocene delta of Orosei, on the east coast of Sardinia, has been dated as Helvetian (Middle Miocene), and interpreted as marking the initiation of the north-south horst of eastern Sardinia[76]. Thus we suggest that the separation of Calabria from Sardinia took place in the middle Miocene, and that Tortonian deformation in Sicily[50] indicates a collision that terminated this phase of separation. The part of the Tyrrhenian Sea west of the Anzio–La Galite scarp would have formed during this third, pre-Messinian, episode of movement and would then have been the site of Messinian evaporite deposition.

The fourth episode occurred from the Pliocene to the Recent, with Calabria moving ESE to its present position. The movement of the Calabrian microplate may be reflected by greater fragmentation of the allochthonous Apennine carbonate block mountains from NW to SE: in the central Apennines the allochthonous masses average 30 × 100 km; in the southern Apennines they are smaller, averaging 10 × 40 km. The big central volcanoes of the SE Tyrrhenian, M. Vavilov and M. Marsili, are probably related to the motion of Calabria. They are located on the WNW–ESE axis of the basin along the track probably followed by Calabria (the up-dip direction of the present seismic zone) and are elongated at right angles to the basin axis[43,44]. Dredge samples show that the volcanic rocks of M. Marsili are tholeiitic and have affinities with those of mid-ocean ridges[77]. Also noteworthy is the remarkably circular pattern of the south-easternmost Tyrrhenian, with the inner ring of the Aeolian Islands–M. Palinuro and the outer ring of the southern Apennines–Calabria–northern Sicily mountains concentric about the Marsili volcano. These observations suggest that M. Marsili and M. Vavilov may mark centres of accretion in a complex, non-linear pattern of behind-the-arc seafloor spreading.

Continuing activity is shown by the high heat flow of the Tyrrhenian Sea[78] and by the active seismic zone dipping WNW beneath Calabria and the associated volcanoes of the Aeolian Islands[37–40]. This narrow seismic zone must be interpreted as showing ESE-directed overriding of a small Calabrian plate, rather than general subduction of Africa beneath Europe[37].

Relation to magnetic studies

Bayer *et al.*[79] give a magnetic anomaly map of the western Mediterranean and a tectonic interpretation based on this

map. Their interpretation agrees with ours in placing the Kabylie massifs among the Mediterranean microplates but differs by placing the poles of rotation of Corsica and Sardinia relative to Europe about 60° away so that there is little change in orientation of the islands during their movement. We have used poles nearby so that the islands have large total angular rotations: 32° (Corsica) and 65° (Sardinia)[4]. The palaeomagnetic results showing large rotations of Corsica[80] and Sardinia[81] are dismissed by Bayer *et al.*, who note that the islands must first have rotated with Iberia; they attribute the 30° palaeomagnetic declination change in Corsica to this Iberian rotation and state that the larger rotation of Sardinia is not compatible with their data. But the Permian declinations from Corsica differ from those of the Esterel Massif in Provence[80], which must also have rotated with Iberia since the north Pyrenean transform cannot be drawn between Corsica and the Esterel in the pre-Miocene reconstruction without destroying the striking geological match[45,80]. Thus the palaeomagnetic data indicate that the islands have rotated with respect to southern France.

We consider the interpretation of the magnetic anomalies to be open to some doubt because of the low amplitude and ambiguous pattern of the anomalies and the inherent problem of correlating the indistinct magnetic profile between anomalies 5 and 6 (10–20 m.y.—the probable age of the Corsica-Sardinia movements) across transform faults. In view of the apparent absence of well developed symmetrical anomaly patterns in marginal basins[82] particular caution should be exercised in interpreting the anomalies of the western Mediterranean, which shows a number of the characteristics of marginal basins. Herron *et al.*[83] emphasise that completely erroneous interpretations can result from incautious use of marine magnetic data. Careful re-examination of both the palaeomagnetic results and the marine magnetic data from the western Mediterranean is clearly necessary.

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[1] Alvarez, W., *Nature phys. Sci.*, **235**, 103 (1972).
[2] Westphal, M., Bardon, C., Bossert, A., and Hamzeh, R., *Earth planet. Sci. Lett.*, **18**, 37 (1973).
[3] Choukroune, P., LePichon, X., Seguret, M., and Sibuet, J. C., *Earth planet. Sci. Lett.*, **18**, 109 (1973).
[4] Alvarez, W., in *Implications of Continental Drift to the Earth Sciences*, **2**, 893, Academic Press, 1973.
[5] *Proc. Congr. Tectonics Sardinia Cagliari*, 1973, (edit. by Pomesano Cherchi, A.) *Rend. Sem. Fac. Sc. Univ. Cagliari* (in the press).
[6] DeJong, K. A., Manzoni, M., Stavenga, T., Van Dijk, F., Van der Voo, R., and Zijderveld, J. D. A., *Nature*, **243**, 281 (1973).
[7] Manzoni, M., in *Geology of Italy*, (Petr. expl. Soc. Libya, Tripoli, in the press).
[8] Alvarez, W., in *Proc. Congr. Tectonics Sardina Cagliari*, 1973, *Rend. Sem. Fac. Sc. Univ. Cagliari* (in the press).
[9] Abbate, E., and Sagri, M., *Sediment. Geol.*, **4**, 251 (1970).
[10] Boccaletti, M., Elter, P., and Guazzone, G., *Nature phys. Sci.*, **234**, 108 (1971).
[11] Maxwell, J. C., *N.Y. Acad. Sci. Trans.*, **21**, 269 (1959).
[12] Giannini, E., and Lazzarotto, A., in *Geology of Italy*, (Petr. expl. Soc. Libya, Tripoli, in the press).
[13] Dubois, R., *Rev. Géogr. Phys. Géol. Dyn.*, **12**, 221 (1970).
[14] Truillet, R., thesis, Univ. Paris (1968).
[15] Truillet, R., in *Geology and History of Sicily*, **171** (Petr. expl. Soc. Libya, Tripoli, 1970).
[16] Nardi, R., Puccinelli, A., and Verani, M., *Mem. Soc. Geol. Ital.*, **10**, 191 (1971).
[17] Nardi, R., *Mem. Soc. Geol. Ital.*, **7**, 323 (1968).
[18] Spijer, S. B., thesis, Univ. Amsterdam (1955).
[19] Delcey, R., Meunier, A., and Routhier, P., *C.r. hebd. Séanc. Acad. Sci.*, **260**, 6644 (1965).
[20] Amaudric du Chaffaut, S., Caron, J. M., Delcey, R., and Lemoine, M., *C.r. hebd. Séanc. Acad. Sci.*, **275**, 2611 (1972).
[21] Caire, A., Glangeaud, L., and Grandjacquet, C., *Bull. Soc. Géol. Fr.*, **2**, 915 (1960).
[22] Piccarreta, G., and Zirpoli, G., *Rend. Soc. Ital. Min. Pet.*, **25**, 121 (1969).
[23] Piccarreta, G., and Zirpoli, G., *Boll. Soc. geol. Ital.*, **88**, 469 (1969).
[24] De Roever, E. W. F., thesis, Univ. Amsterdam, 171 p. (1972).
[25] Hoffmann, C., *Contr. Mineral. Petrol.*, **27**, 283 (1970).
[26] Brouwer, H. A., and Egeler, C. G., *Kon. Ned. Ak. Wet., Verh. Afd. Nat. (Tweede Reeks)*, **48**, 1 (1952).
[27] Bearth, P., *Schweiz. Mineral. Petrog. Mitt.*, **46**, 13 (1966).
[28] Nijhuis, H. J., thesis, Univ. Amsterdam, 151 p. (1964).
[29] Wezel, F. C., in *Geology in Italy*, (Petr. expl. Soc. Libya, Tripoli, in the press).
[30] Wezel, F. C., and Ryan, W. B. F., *Boll. Soc. geol. Ital.*, **90**, 249 (1971).
[31] Wezel, F. C., *Riv. Min. Siciliana*, **21**, 187 (1970).
[32] Burckhardt, C. E., *La Ricerca Scientifica*, **22**, 86 (1952).
[33] Caire, A., in *Geology and History of Sicily*, **145**, (Petr. expl. Soc. Libya, Tripoli, 1970).
[34] Bossière, G., and Raymond, D., *C.r. hebd. Séanc. Acad. Sci.*, **275**, 641 (1972).
[35] Durand-Delga, M., *Publ. Serv. Géol. Algérie*, **39**, 89 (1969).
[36] Caire, A., in *Tectonique de l'Afrique* (Sciences de la Terre, 6), Unesco, **61** (1971).
[37] Ritsema, A. R., *Verh. Kon. Ned. Geol. Mi jnb. Gen.*, **26**, 105 (1969).
[38] Caputo, M., Panza, G. F., and Postpischl, D., *J. geophys. Res.*, **75**, 4919 (1970).
[39] Ninkovich, D., and Hays, J. D., *Acta int. Congr. Volcano Thera*, **111** (1971).
[40] Barberi, F., Gasparini, P., Innocenti, F., and Villari, L., *J. geophys. Res.*, **78**, 5221 (1973).
[41] Chabrier, G., *C.r. hebd. Séanc. Acad. Sci.*, **271**, 1252 (1970).
[42] Alvarez, W., and Cocozza, T. C., *Rend. Sem. Fac. Sc. Univ. Cagliari* (in the press).
[43] Morelli, C., *Boll. Geofis. Teor. Appl. (Trieste)*, **12**, 275 (1970).
[44] Selli, R., and Fabbri, A., *Rend. Acc. Naz. Lincei, Rend. Cl. Sc. mat. fis. nat.*, **50**, 104 (1971).
[45] Ryan, W. B. F., Hsü, K. J., *et al., Init. Rep. Deep Sea Drilling Project*, **13** (1) (US Government Printing Office, Washington, DC, 1972).
[46] Vardabasso, S., *Rend. Acc. Naz. Lincei, Rend. Cl. Sc. mat. fis. nat.*, **14**, 8, 178 (1953).
[47] Wezel, F. C., and Guerrera, F., *Boll. Soc. geol. Ital.*, **92**, 193 (1973).
[48] Wezel, F. C., *Mem. Soc. geol. Ital.*, **7**, 417 (1968).
[49] Wezel, F. C., *Nature*, **228**, 275 (1970).
[50] Wezel, F. C., *Mem. Soc. geol. Ital.*, **9**, 225 (1970).
[51] Coiffait, P. E., *C.R. Somm. Soc. geol. Fr.*, **79** (1972).
[52] Dewey, J. F., Pitman, W. C. III, Ryan, W. B. F., and Bonnin, J., *Bull. geol. Soc. Am.*, **84**, 3137 (1973).
[53] Egeler, C. G., and Simon, O. J., *Geol. en Mijnb.*, **48**, 296 (1960).
[54] Egeler, C. G., and Simon, O. J., *Kon. Ned. Ak. Wet., Verh. Afd. Nat. (Eerste Reeks)*, **25**, 1 (1969).
[55] Afchain, C., *Bull. Soc. geol. Fr.*, **4**, 719 (1962).
[56] Durand-Delga, M., in *Guidebook to the Geology and History of Tunisia, Petr. Expl. Soc. Libya, Tripoli*, **59** (1967).
[57] Pitman, W. C. III, and Talwani, M., *Bull. geol. Soc. Am.*, **83**, 619 (1972).
[58] Rutten, M. G., *The Geology of Western Europe* (Elsevier, 1969).
[59] Bousquet, J. C., Thèse, Montpellier (1972).
[60] Boccaletti, M., and Guazzone, G., *Mem. Soc. geol. Ital.*, **11**, 201 (1972).
[61] Auzende, J. M., Bonnin, J., and Olivet, J. L., *Q. Jl. geol. Soc. Lond.*, **129**, 607 (1973).
[62] Karig, D. E., *J. geophys. Res.*, **76**, 2542 (1971).
[63] Schäffer, K., and Cocozza, T., *Rend. Sem. Fac. Sc. Univ. Cagliari* (in the press).
[64] LePichon, X., Pautot, G., Auzende, J. M., and Olivet, J. L., *Earth planet. Sci. Lett.*, **13**, 145 (1971).
[65] Pecorini, G., and Pomesano Cherchi, A., *Mem. Soc. geol. Ital.*, **8**, 421 (1969).
[66] Cocozza, T., in *Modello Strutturale d'Italia, Illustrative Volume*, (CNR, Rome, in the press).
[67] Baldacci, F., Elter, P., Giannini, E., Giglia, G., Lazzarotto, A., Nardi, R., and Tongiorgi, M., *Mem. Soc. geol. Ital.*, **6**, 213 (1967).
[68] *Service Carte Géologique France*, Sheet 266–267 (1:80,000), *Notice explicative* (1962).
[69] Pomesano Cherchi, A., in *Proc. fifth Congr. Mediterranean Neogene, (Lyons, 1971,* in the press).

[70] Blow, W. H., *Proc. first int. Conf. Planktonic Microfossils*, **1**, 199 (1969).
[71] Auzende, J. M., *Mar. geophys. Res.*, **1**, 162 (1971).
[72] Auzende, J. M., Olivet, J. L., and Bonnin, J., *Tectonophysics* (in the press).
[73] Van Bemmelen, R. W., *Verh. Kon. Geol. Mijnb. Gen.*, **26**, 13 (1969).
[74] *Giornale di Geologia (Bologna)*, **37** (edit. by Selli, R.) (1970).
[75] Selli, R., *Mem. Soc. geol. Ital.*, **3**, 737 (1962).
[76] Dieni, I., and Massari, F., *Mem. Soc. Ital. Sci. nat.*, **15**, 91 (1966).
[77] Del Monte, M., *Giorn. geol. (Bologna)*, **38**, 231 (1972).
[78] Erickson, A., thesis, MIT (1970).
[79] Bayer, R., LeMouel, J. L., and LePichon, X., *Earth Planet. Sci. Lett.*, **19**, 168 (1973).
[80] Nairn, A. E. M., and Westphal, M., *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, **5**, 179 (1968).
[81] Zijderveld, J. D. A., DeJong, K. A., and Van der Voo, R., *Nature*, **226**, 933 (1970).
[82] Karig, D. E., *J. geophys. Res.*, **76**, 2542 (1971).
[83] Herron, E. M., Dewey, J. F., and Pitman, W. C. III, Geol. Soc. Am., *Abstracts with Programs*, **5**, 666 (1973).

Size and folding of the messenger for phage T4 lysozyme

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The existence of non-random base pairing in T4-specific mRNA is supported by the nuclease digestion patterns of the messenger for phage T4 lysozyme. Under conditions which denature RNA, the nuclease nicked lysozyme mRNA recovers its activity. This behaviour can be explained by a model in which secondary structure controls initiation at existing ribosomal attachment sites.

We have reported techniques for assaying the biological activity of lysozyme messenger RNA in an *in vitro* system[1], and by use of this technique were able to show in preliminary studies[2,3] that the lysozyme mRNA activity is found in molecules much larger than the lysozyme structural gene alone.

We now show that lysozyme mRNA activity extracted from infected cells occurs in molecules of several discrete sizes. The lysozyme messenger activity of the larger molecules can be destroyed by treatment with T1 RNase *in vitro*. These inactivating cleavages can occur at specific locations outside the structural gene for lysozyme as shown by the fact that, on heat disaggregation, biological activity (for lysozyme synthesis) is recovered in smaller molecules of discrete size classes. We have previously shown by several independent methods that T4-specific mRNAs contain a large amount of highly ordered base-pairing (submitted for publication.) The experiments described here provide further evidence that T4 mRNA molecules have a well defined secondary structure and we discuss the implications of this evidence for processing of bacteriophage mRNA molecules *in vivo* and for control of translation by mRNA secondary structure.

Translation of lysozyme mRNA fragments

RNA extracted 25 min after infection of *E. coli* B^E with phage T4D+ can code for lysozyme synthesis in an *in vitro* system[1,2]. Fig. 1 shows that digestion with increasing amounts of RNase T1 progressively destroys this ability. To analyse the effects of such digestions on the sizes of the surviving lysozyme mRNA molecules, we have sedimented such preparations on sucrose gradients (Fig. 2). Before nuclease treatment, the RNA was heated at 80° C for 2 min in a low ionic strength buffer containing 0.01 M EDTA, 0.01 M Tris-Cl, *p*H 7.4 (measured at room temperature), then quick-cooled. The purpose of this procedure, 'heat disaggregation', was to dissociate any aggregates and to reveal any pre-existing 'hidden breaks'. The preparation was then sedimented either without digestion (Fig. 2*a*), with digestion (Fig. 2*b* and *d*), or with digestion followed by a second heat disaggregation (Fig. 2*c* and *e*).

In the undigested control sample (Fig. 2*a*), lysozyme mRNA activity sediments in discrete multiple peaks. Other experiments involving sedimentation in denaturing solvents indicate that active lysozyme mRNA exists in a number of discrete size classes (our unpublished results). Although RNase T1 digestion inactivates the lysozyme mRNA, the activity has a sedimentation profile similar to the control and, in particular, there is no indication that activity is shifted to slower sedimenting peaks (compare Fig. 2*b* and *d* with Fig. 2*a*).

This is the result expected if lysozyme mRNA is tightly folded with a great deal of base pairing. In such a situation, many of the single-strand breaks would be masked so that a lysozyme mRNA molecule containing several nuclease cleavages could be held together by base-pairing, and sediment as a single unit. The results in Fig. 2*c* and *e* show that the nuclease-treated molecules do contain hidden breaks: if the nuclease treatment is followed by a second heat-disaggregation these breaks are revealed and the lysozyme mRNA activity is found in smaller fragments sedimenting at about 13S and 18S (compare Fig. 2*c* with Fig. 2*b* and Fig. 2*e* with Fig. 2*d*). This suggests that the lysozyme mRNA has a well-defined secondary structure in which a small number of very susceptible regions ('hotspots') are preferentially cleaved. If this were not the case, then we would expect the nuclease treatment to yield a broad distribution of fragment sizes with no definite peaks.

Prolonged heating at 80° C destroys lysozyme messenger activity (unpublished data of B. R.). For our heat disaggregation steps, we have used very short heating times to minimise the amount of thermal inactivation. Since Möller and Boedtker[5] have suggested that heating causes chain breaks, there remained the possibility that thermal chain breaks might explain some of our results. To test this, we have investigated the effect of heating for various lengths of time on the sedimentation pattern. The results show that the main effect of heating is to disaggregate and reveal hidden breaks: the changes in sedimentation pattern which do occur on heating occur very rapidly and are independent of the time of the heat treatment. Thermal breaks, if they occur, should continue to accumulate throughout the heat treatment. After 10 min of heat treatment, which inactivated 74% of the lysozyme messenger activity, there was no indication of thermal breaks in either the sedimentation profile of lysozyme mRNA activity or in the ultraviolet absorption profile of

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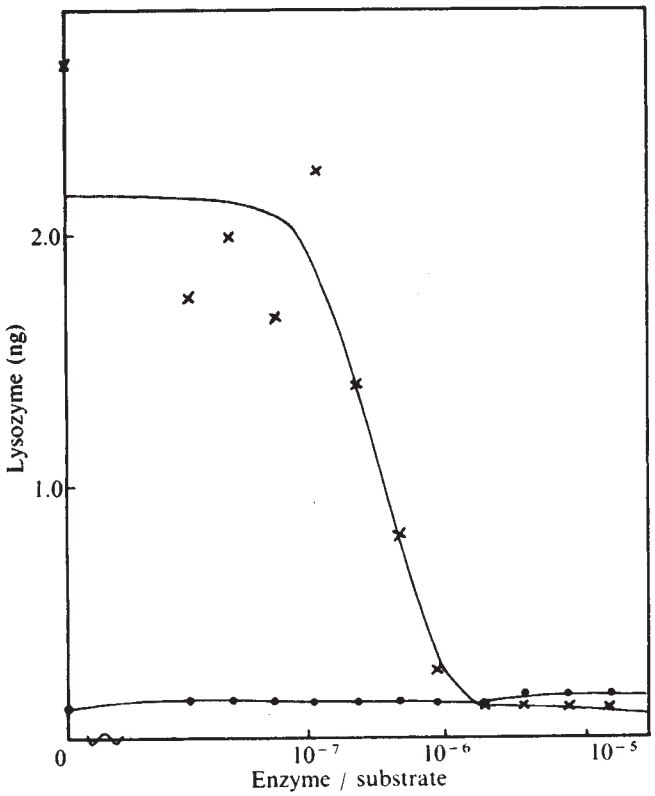


FIG. 1 130 μg RNA, extracted either 5 min (●) or 25 min (X) after infection of *E. coli* B^s with T4D$^+$, at a concentration of 4.8 mg ml^{-1} was treated with RNase T1 in 0.2 M NaCl, 0.02 M magnesium acetate, 0.05 M Tris-Cl, pH 7.5, in the presence of 50 μg ml^{-1} bovine serum albumin (BSA). The largest enzyme to substrate concentration was 1/130,000. After a 10 min incubation at 37° C, EDTA was added in stoichiometric amounts to the Mg^{2+} and the RNA tested in a protein synthesising system *in vitro*.

rRNA. Apparently, most of the thermal inactivation of mRNA which we see must be due to some process which is considerably more rapid than thermal chain breakage, although much slower than heat disaggregation.

Heating activates nicked mRNA

Not only does heat disaggregation cause nuclease-treated lysozyme mRNA activity to shift from rapidly sedimenting complexes to smaller fragments, but also some of the lysozyme messenger activity which was originally lost in the nuclease treatment is recovered (Fig. 2). If we take the original lysozyme messenger activity to be 100%, then the preliminary heat disaggregation reduces the activity to 72%. Subsequent nuclease treatment at an enzyme to substrate ratio of 1:3.2 × 10^6 (w/w) causes the activity to decrease to 40% of the original. But a second heat disaggregation causes a 1.4-fold increase in the activity, to 56% of the original. With twice as much nuclease the enzyme activity drops to 21%, but the second heat disaggregation causes a 1.55-fold increase in activity, to 32.5% of the original activity.

These increases in enzyme activity are even more impressive when we consider that the normal effect of the heat treatment alone is to cause a roughly 21% inactivation of messenger activity (unpublished results of B.R.). Corrected for this factor the 1.4-fold activation noted above would be a 1.77-fold increase in activity and the 1.55-fold activation would be a 1.96-fold increase in activity. The stimulation of ability to make active lysozyme resulting when nuclease-treated mRNA was disaggregated was confirmed in other experiments involving heat treatments and immediate assay without sedimentation on sucrose gradients. One sort of model which can explain this type of result is shown in diagrammatic form in Fig. 3. In this hypothetical model it is

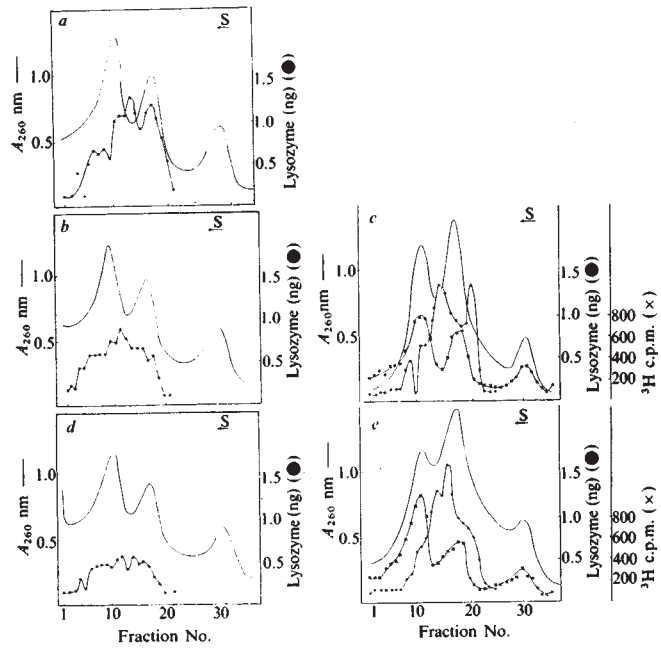


FIG. 2 *a*, One mg 25 min RNA was heat disaggregated, adjusted to 1 ml in 0.1 M NH_4Cl, 0.01 M ammonium acetate, *p*H 4.6 and layered on a 36 ml 5–20% sucrose gradient made with RNase-free sucrose in the same buffer. Centrifugation was in a Spinco SW27 rotor at 27,000 r.p.m. for 17 h at 4° C. Fractions of 1 ml were collected and the RNA was precipitated with 2 volumes cold ethanol and 0.1 volumes of 3 M sodium acetate, *p*H 5.1. The RNA was left at −20° C overnight and the precipitated RNA was pelleted in a clinical centrifuge at top speed. The pellets were allowed to drain for 1–2 h at 4° C[8] and then were dissolved in 50 μl of sterile, doubly distilled water. A sample of 25 μl was assayed for lysozyme mRNA activity. The recovery of activity was 72%. Note that in *a*, as well as in *b* and *d*, in which there was only a single heat disaggregation step, there is still some aggregation of rRNA (as shown by ultraviolet-absorbing material sedimenting faster than 23S). We believe that the sedimentation pattern which we observe for the lysozyme mRNA activity is not due to aggregation since the peaks observed after heat disaggregation are similar to those observed during sedimentation in denaturing solvent (DMSO gradients) or when the mRNA has been partially purified before centrifugation so that aggregation with rRNA cannot occur.[2] In any case, the most important conclusions which we wish to draw are based on changes in the sedimentation profiles of active lysozyme mRNA after the various treatments and these conclusions are largely independent of whether some aggregation is occurring under the sedimentation conditions used. ——, $A_{260\,nm}$; ● —— ●, ng lysozyme synthesised. *b*, One mg 25 min RNA which had been heat disaggregated was digested with RNase T1 as in Fig. 1 and layered into a sucrose gradient without further heat treatment. The enzyme to substrate ratio was 1:3.2 × 10^6. After sucrose gradient analysis the recovery of activity was 40% of the original. ——, $A_{260\,nm}$; ●——●, ng lysozyme synthesised. *c*, One mg 25 min RNA was heat disaggregated and digested with RNase T1 as in (*b*). Then, to unmask hidden breaks, the RNA was precipitated (by adding 2 volumes ethyl alcohol and 0.1 volumes 3 M sodium acetate, *p*H 5.1 and centrifuging after 30 min at −20° C), resuspended in 0.5 ml 0.01 M Tris-Cl, *p*H 7.4, 0.01 M EDTA, and again heat disaggregated. The RNA was then layered onto a sucrose gradient and centrifuged as in (*a*) with ^{3}H-labelled stable RNA added to provide sedimentation markers. The recovery of activity was 56% of the original. ——, $A_{260\,nm}$; ●——●, ng lysozyme synthesised; X——X, ^{3}H-labelled stable RNA markers. *d*, One mg 25 min RNA was heat disaggregated, then digested with RNase T1 at an enzyme to substrate ratio of 1:1.6 × 10^6. After sucrose gradient analysis, the recovery of activity was 21% of the original. ——, $A_{260\,nm}$; ●——●, ng lysozyme synthesised. *e*, One mg 25 min RNA was heat disaggregated and digested with RNase T1 as in (*d*), then reheated in low salt to unmask hidden breaks and centrifuged with ^{3}H-labelled stable RNA markers as described in (*c*). The recovery of activity was 32.5% of the original. ——, A_{260nm}; ●——●, ng lysozyme synthesised; X——X, ^{3}H-labelled stable RNA markers.

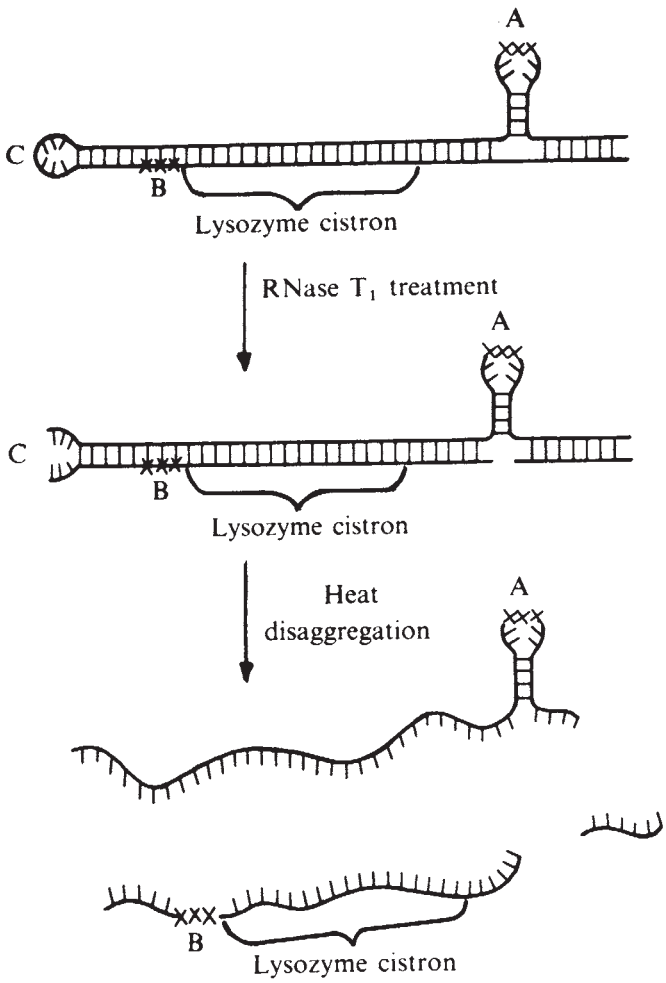


FIG. 3 Model showing how heating could reactivate nuclease-inactivated mRNA by exposing base-paired ribosomal attachment sites.

imagined that the rapidly sedimenting lysozyme messengers contain (at least) two ribosomal attachment sites, A and B. Lysozyme synthesis is carried out by ribosomes which attach at ribosomal attachment site A, which is exposed, and continue on into the lysozyme cistron. Ribosomes cannot attach at site B because it is in a base-paired configuration. RNase, by cleaving the loop at C prevents the ribosomes from continuing from site A into the lysozyme cistron but ribosomal attachment site B is still blocked by base-pairing. This nuclease-treated mRNA is consequently incapable of directing lysozyme synthesis. By heat disaggregation, however, a small mRNA fragment is liberated which can be translated to yield lysozyme since ribosomal attachment site B is no longer masked by base-pairing.

Processing of mRNA?

Aggregation artefacts cannot explain the fact that the lysozyme mRNA activity extracted from T4-infected cells sediments with a definite peak at about 18S and a shoulder at about 13S (Fig. 4). Corresponding peaks occur under conditions which prevent aggregational artefacts (sedimentation in denaturing solvents or after removal of the stable RNA species) and in dimethyl sulphoxide about 39% of the mRNA activity sediments even faster than the main peak (1,700 nucleotides long, our unpublished results). Since all of the lysozyme mRNA activity is known by several criteria to come from a single structural gene[6], we are forced to one of two possible conclusions. The first possibility is that the primary transcripts are themselves made in a variety of sizes, either because RNA polymerase transcribes lysozyme mRNA from a number of different initiation points or because transcription stops at a number of 'partial' termination sites. The second possibility is that the lysozyme mRNA is transcribed as part of a very large molecule, at least 3,000 nucleotides long, and that all the smaller species are produced by endonucleolytic cleavages. Such cleavages could occur *in vivo*, in which case they represent natural mRNA processing steps, or they could occur during the extraction of the messenger (although this is carried out under conditions —sodium dodecyl sulphate, hot phenol—which should minimise nuclease action).

Of course the possibilities above are not mutually exclusive and each could play a role. Of the two we favour the second (processing type cleavage of a large precursor molecule) since the same size (13S and 18S) fragments can be obtained *in vitro* from the large mRNA molecules by RNase T1 digestion.

Attempts to activate 5′ lysozyme mRNA

As previously reported, mRNA extracted 5 min after T4 infection of *E. coli* (5′ mRNA) is not competent for lysozyme synthesis in an *in vitro* protein synthesising system[2]. Nevertheless, both hybridisation[7] and transformation[8] experiments have demonstrated that lysozyme mRNA is physically present at 5 min. The presence of factors early in infection which might inactivate early lysozyme mRNA and the possibility that the 'early' ribosomes are incompetent have been ruled out already[2]. Our present experiments indicating that secondary structure can prevent translation of lysozyme mRNA support the idea that lysozyme mRNA might always be made in a form inactive due to its secondary structure. Late in infection such a lysozyme mRNA could be activated, perhaps by cleavage of a fragment which exposes the ribosomal attachment site or by modification of the structure.

With this in mind, we attempted the following experiments to activate 5′ mRNA. To determine whether we could activate early lysozyme mRNA by adding some factor produced late in infection we replaced the S30 extract from uninfected *E. coli* with an S30 extract prepared from cells infected for 30 min with T4em74, a T4 mutant carrying a nonsense codon in the lysozyme *e* gene. No lysozyme synthesis was obtained with 5′ mRNA even at the magnesium concentration which

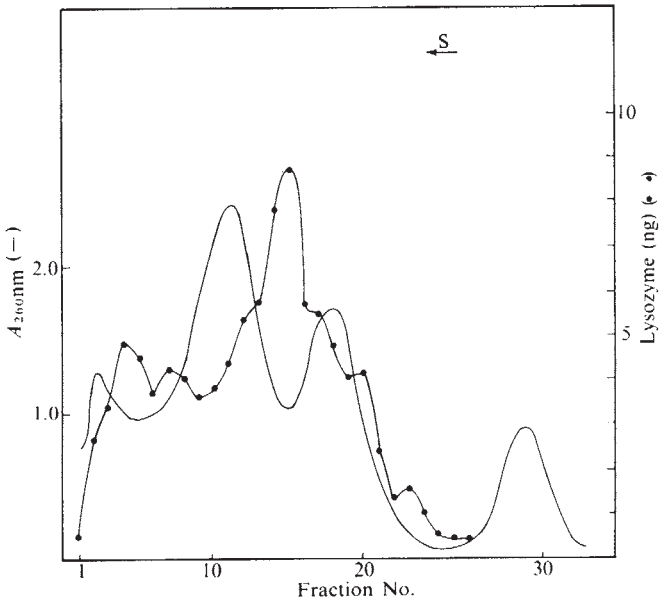


FIG. 4 Two mg RNA extracted with sodium dodecyl-sulphate and hot phenol from *E. coli* infected with T4D^{+1} was layered onto a sucrose gradient, as described in Fig. 2. The recovery of activity was 96%. ——, $A_{260\,nm}$; ● ——●, ng lysozyme synthesised.

gave optimal synthesis with the late mRNA control. Attempts were also made to activate the early lysozyme mRNA by carrying out the *in vitro* synthesis at elevated temperatures or by limited digestion of the early mRNA with RNase T1. The RNase T1-treated material was tested both with and without a subsequent heat disaggregation. No evidence for activation of 5′ lysozyme mRNA was obtained even though the controls carried out with 25′ mRNA indicated that we had succeeded in covering the optimal range of each one of these treatments.

Base-pairing in lysozyme mRNA non-random

It has long been established that the mRNA from the RNA bacteriophages is highly base-paired. Recent work with f2 and R17 RNA suggests that the secondary structure of these molecules is related to translational control[9,10]. But the RNA phage genomes may be unlike other mRNA species since they are self-replicating and must be capable of folding to fit a spherical virus capsid: their secondary structure might be for instance, a special adaptation to facilitate encapsulation[11,12]. For this reason we have examined bacteriophage T4 messenger RNAs. Since they are synthesised from a double stranded DNA genome their characteristics may be more nearly typical of other mRNA species.

Our previous experiments utilising several indirect methods (optical studies, partial digestion with single-strand specific nucleases, and sedimentation under denaturing and non-denaturing conditions) suggested that T4-specific mRNAs contain a considerable amount of base pairing (submitted for publication). But Fresco *et al.*[13] have asserted that even random RNA sequences may contain as much as 60% base pairing. If the random base pairing structures they proposed are compared directly with those derived from direct sequencing of 16S rRNA[14,15] it becomes immediately obvious that the random structures should have much lower melting temperatures, less cooperativity in their melting curves and greater sensitivity to single-strand-specific nucleases. Since our previous experiments show that T4 mRNAs are very similar to rRNA in all these regards, (and unlike random copolymers, our data in preparation) it seems clear that chance base pairing cannot explain the secondary structure observed.

The idea that the secondary structure of lysozyme mRNA is highly ordered is further supported by our finding that nuclease treatment yields active fragments in discrete size classes. The mRNA molecules would be expected to exist in a large number of different configurations with similar stabilities if only random base pairing were involved. If so, nuclease digestion of exposed single stranded regions should not have given the discrete peaks of activity observed in Fig. 2*c* and *e*.

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1 Salser, W., Gesteland, R. F., and Bolle, A., *Nature*, **215**, 558 (1967).
2 Salser, W., Gesteland, R. F., and Ricard, B., *Cold Spring Harbor Symp. quant. Biol.*, **34**, 771 (1969).
3 Gesteland, R. F., and Salser, W., *Genetics*, **61**, 1, (1969).
4 Bolle, A., Epstein, R., Salser, W., and Geiduschek, E. P., *J. molec. Biol.*, **31**, 32.
5 Möller, W., and Boedtker, H., in *Acides Ribonucleiques et Polyphosphates Structure, Synthese et Fonctions*, **106**, 99 (Editions du CNRS, Paris, 1962).
6 Gesteland, R. F., and Salser, W., and Bolle, A., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 203 (1967).
7 Bautz, E. K. F., Kasai, T., Reilly, E., and Bautz, F. A., *Proc. natn. Acad. Sci. U.S.A.*, **55**, 1081 (1966).
8 Jayarman, R., and Goldberg, E. B., *Cold Spring Harbor Symp. quant. Biol.*, **35**, 197 (1970).
9 Lodish, H. F., and Robertson, H. D., *Cold Spring Harbor Symp. quant. Biol.*, **34**, 655 (1969).
10 Fukami, H., and Imahori, K., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 570 (1971).
11 Jeppesen, P. G. N., Nichols, J. L., Sanger, F., and Barrell, G. B., *Cold Spring Harbor Symp. quant. Biol.*, **35**, 13 (1970).
12 Adams, J. M., Jeppesen, P. G. N., Sanger, F., and Barrell, G. B., *Nature*, **223**, 1009 (1969).
13 Fresco, J. R., Alberts, B. M., and Doty, P., *Nature*, **168**, 98 (1960).
14 Fellner, P., Ehresman, C. and Ebel, J. P., *Cold Spring Harbor Symp. quant. Biol.*, **35**, 29 (1970).
15 Ehresman, C., Stiegler, P., Fellner, P., and Ebel, J. P., *Biochemie*, **54**, 901 (1972).

LETTERS TO NATURE

PHYSICAL SCIENCES

Difficulty of interstellar radio communication

In 1959 Cocconi and Morrison[1] suggested that the hyperfine transition of atomic hydrogen at 1,420.405 MHz is the uniquely rational choice of frequency to use in attempts at interstellar communication. Since then other rational choices have been put forward: for example, the first or second overtones of this frequency[2,3] or the frequency of one of the hydroxyl transitions at 1,612, 1,665, 1,667 and 1,720 MHz[3,4]. Very recently Drake and Sagan[5] have argued ingeniously in favour of yet two further frequencies. Unfortunately, none of the rational choices may now be treated with confidence as the correct one. This magnifies the difficulty of the problem.

By an understandable process of self-selection, writers on the subject of interstellar radio communication tend to be at least cautiously optimistic. The view of the perhaps rashly pessimistic should not be allowed to suffer by default.

Following Drake and Sagan[5] I shall for definiteness make what they refer to as "the rather optimistic assumption" that the number of stars n_s which must be searched is about 10^6, which corresponds to the number of civilisations N in the Galaxy being about 10^5 and to the likely distance to the nearest civilisation being several hundred (say 300) light years. In the steady state[6]

$$N = RL \qquad (1)$$

where R is the annual rate at which civilisations evolve and L is their average lifetime. It has been estimated[3] that R may be as high as 10^{-1} yr^{-1}, in which case equation (1) may be written

$$N = 10^{-1}L \qquad (2)$$

and for N to be 10^5 would require L to be 10^6 yr.

The probability of a transmitting radio telescope on a

distant planet (civilisation X) and a receiving radio telescope on Earth (civilisation E) being directed toward one another at corresponding times is minute. Sophisticated survey schemes may be envisaged but may be ignored in the context of an order of magnitude calculation.

To favour the feasibility of the civilisations making radio contact, I shall suppose that both X and E take their orbital motion around their parent star accurately into account, and I shall suppose further that the presumably technically very much more advanced X measures the radial velocity of every star in the survey to within ± 0.01 km s^{-1}, as seems possible[7], and also takes this into account. At the frequency of the hyperfine line of atomic hydrogen (which for definiteness I shall use for illustrative purposes) the uncertainty in the Dopplar shift would then be about 10^2 Hz. To keep the number of channels as few as possible I shall suppose that X transmits on a band of this width. The power radiated would have to be high. For example, if the dishes of both radio telescopes were 300 m in diameter, some 10 MW would be needed to reach a distance of 300 light years with a strength equal to sky noise at a temperature as low as 10 K. Finally, to favour the feasibility of the venture under consideration yet again, I shall suppose that E guesses X's general procedure (and is content to use a single channel).

The times required for X and E to survey n_s stars systematically are respectively

$$J(\mathrm{X}) = n_s n_c \tau(\mathrm{X}) \tag{3}$$

and

$$J(\mathrm{E}) = n_s \tau(\mathrm{E}) \tag{4}$$

where n_c is the number of channels regarded as rational choices and where $\tau(\mathrm{X})$ and $\tau(\mathrm{E})$ are the X and E times per star per channel.

Sending a significant signal takes longer than detecting the existence of a signal, so

$$\tau(\mathrm{X}) > \tau(\mathrm{E}) \tag{5}$$

and hence

$$J(\mathrm{X}) > J(\mathrm{E}) \tag{6}$$

Accepting equations (5) and (6) it is a straightforward task to prove that during any complete survey of the n_s stars by X the probability that two dedicated radio telescopes would be directed towards one another at corresponding times when using the same channel is

$$P = \tau(\mathrm{X})/J(\mathrm{E}) \tag{7}$$

provided

$$\tau(\mathrm{X}) < J(\mathrm{E}) \tag{8}$$

Omitting the time of passage between X and E, the time before radio contact is likely to be established is therefore around

$$T = J(\mathrm{X})/P \tag{9}$$

$$= n_s^2 n_c \tau(\mathrm{E}) \tag{10}$$

Subject to condition (8) being satisfied, T is independent of $\tau(\mathrm{X})$ so that quite an extended message could in principle be transmitted.

In view of the suggestions referred to in the first paragraph, I think it not unreasonable to assign the value 4 to n_c, in which case (10) may conveniently be written

$$T = 10^5 \tau(\mathrm{E}) \text{ yr} \tag{11}$$

where $\tau(\mathrm{E})$, which of course includes the time lost in changing from one star to the next, is measured in s. It was the practice to observe a band 100 Hz wide for 60 s in Project Ozma. Though $\tau(\mathrm{E})$ could certainly be made much briefer than this, it is apparent from equation (11) that T is a depressingly long time. The position would naturally be eased if X and E were each sufficiently concerned to use a number of dedicated radio telescopes. Some adverse factors not mentioned here have to be taken into consideration (see ref. 8).

Agreeing with the penultimate sentence of the previous paragraph the referee has argued that there is no real problem because X is likely to use as many as 10^5 dedicated radio telescopes, the cost being of little moment owing to X's highly advanced technology. This is indeed conceivable (though if the population of X were, say, 10,000 million it would correspond to one dedicated radio telescope per hundred thousand inhabitants). I think, however, that nothing can be said with confidence about the true wealth of X, which is determined by many complex factors such as the balance between the effects of technical progress and depletion of natural resources. Again I think it is open to question whether X's interest in galactic anthropogeny would be deep and long lasting.

An entirely different time is now of relevance. How long would a search project be continued in the absence of success? I am sceptical that it would be continued for even a century but adopting a very favourable view I shall suppose that it would continue for 500 yr. Furthermore, I shall suppose that the complete failure of one search project would not prevent another being initiated at some later date. Repeated failures would make fresh starts ever more difficult and 10^4 yr seems to me a very generous upper limit to the average total duration of the searches, that is to the average lifetime of the civilisation in communicative phases. This increases the effective extent of our isolation.

Referring to equation (2) it may be seen that a consequence is that the number of communicative civilisations in the Galaxy is at most 10^3, from which it follows that the likely distance to the nearest is at least several thousand light years. Perhaps most serious, the value of the right side of formula (11) for the time before contact between a pair of dedicated radio telescopes is likely to be made becomes 10^7 $\tau(\mathrm{E})$ years or, more probably, very much longer (some of the favourable suppositions made in the derivation of the formula being now untenable). The prospect is unpromising.

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1 Cocconi, G., and Morrison, P., *Nature*, **184**, 844 (1959).
2 von Hoerner, S., in *Interstellar Communication* (edit. by Cameron, A. G. W.) 272 (Benjamin, New York, 1963).
3 Shklovskii, I. S., and Sagan, C., *Intelligent Life in the Universe* (Holden-Day, San Francisco, 1966).
4 Barrett, A. H., *Science*, **157**, 881 (1967).
5 Drake, F. D., and Sagan, C., *Nature*, **245**, 258 (1973).
6 Drake, F. D., *Discussion at Space Science Board—National Academy of Sciences, Conf. Extraterrestrial Life, Greenbank, West Virginia* (1961).
7 Griffin, R., and R., *Mon. Not. R. astr. Soc.*, **162**, 243 (1973).
8 Bates, D. R., *Phys. Bull.*, **23**, 26 and 308 (1972).

CH_4 and CH_2 in comets

MANY of the simple radicals observed in cometary spectra, for example OH and NH, probably originate as dissociation products of more stable parent molecules such as H_2O and NH_3. Ip and Mendis[1] have recently discussed the possibilities of observing H_2O in Comet Kohoutek (1973f). If CH_4 exists in significant abundance in comets, it could be a parent of observed CH and CH^+. It is difficult to observe CH_4 directly but a rather specific though indirect means of detecting its presence is suggested.

Dissociation of CH_4 by monochromatic photons at the wavelength of hydrogen Lα (1,216 Å) results in

$$CH_4 + h\nu_{L\alpha} \rightarrow CH_2(\tilde{b}\,^1B_1) + H_2 \qquad (1)$$

(see ref. 2). Fluorescence, resulting from the radiative decay of the excited CH_2 by emission in the $\tilde{b}\,^1B_1$–$\tilde{a}\,^1A_1$ system, accompanies this process. Hydrogen Lα is the strongest emission line in the solar spectrum and its intensity at any point in the orbit of a comet can be calculated. A search for the lines of CH_2 ($\tilde{b}\,^1B_1$–$\tilde{a}\,^1A_1$) lying between wavelengths of 5,000 Å and 9,000 Å (refs 3 and 4) may be a useful, specific probe for both CH_2 and CH_4.

It is safe to assume that there is no other process capable of exciting the $\tilde{b}\,^1B_1$ state of CH_2 efficiently, and that the state is unlikely to be deexcited by collision before radiating. The total emissivity in lines of CH_2, $E(CH_2)$, is related to the concentration (cm^{-3}) of CH_4, $n(CH_4)$ (cm^{-3}), and to the solar Lα flux, $\Phi_{L\alpha}$, according to

$$E(CH_2) = \Phi_{L\alpha} n(CH_4) \sigma(CH_4) \text{ photons cm}^{-3}\text{ s}^{-1}$$

where $\sigma(CH_4)$ is the cross section of process (1), estimated to have a maximum value of about 1×10^{-20} cm^2 (ref. 2). At a distance of 1.0 AU from the Sun, $\Phi_{L\alpha} \cong 3 \times 10^{11}$ photons cm^{-2} s^{-1} (ref. 5), and therefore

$$E(CH_2) \cong 3 \times 10^{-9} n(CH_4) R_{sc}^{-2} \text{ photons cm}^{-3}\text{ s}^{-1}$$

where R_{sc} is the distance (AU) of the comet from the Sun. The total flux in the lines of the $\tilde{b}\,^1B_1$–$\tilde{a}\,^1A_1$ system of CH_2 received at the Earth is given by

$$\Phi(CH_2) \cong 8 \times 10^{-10} R_{sc}^{-2} \int n(CH_4)\, dl \left(\frac{R_{coma}}{R_{ec}}\right)^2 \text{ photons cm}^{-2}\text{ s}^{-1}$$

The integration is along the path length over which CH_4 is in gaseous form in the coma, R_{coma} is an average size describing the region of emission and R_{ec} is the Earth–comet distance.

For Comet Kohoutek (1973f) near perihelion, $R_{sc} \simeq 0.15$ AU, $R_{coma} \gtrsim 10^{11}$ cm and $R_{ec} \simeq 1.0$ AU $= 1.5 \times 10^{13}$ cm, and therefore a flux at the Earth in the CH_2 lines

$$\Phi(CH_2) = 2 \times 10^{-12} \int n(CH_4)\, dl \text{ photons cm}^{-2}\text{ s}^{-1}$$

An effective column density $\int n(CH_4)\, dl$ of 10^{15} cm^{-2} should produce detectable CH_2 emission even if it is distributed over 100 lines of roughly equal strength: approximately 2×10^3 photons s^{-1} per line, assuming a 60 inch telescope with a spectrometer or spectrograph such that the quantum efficiency for the complete system is 0.01. The vacuum wavenumbers of the CH_2 lines which have been observed in absorption are given by Herzberg and Johns[4].

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[1] Ip, W.-H., and Mendis, D. A., *Nature*, **245**, 197 (1973).
[2] Welch, A. R., and Judge, D. L., *J. chem. Phys.*, **57**, 286 (1972).
[3] Herzberg, G., *Proc. R. Soc.*, **A262**, 291 (1961).
[4] Herzberg, G., and Johns, J. W. C., *Proc. R. Soc.*, **A295**, 107 (1966).
[5] Hinteregger, H. E., *Ann. Geophys.*, **26**, 547 (1970).

Sixth radio outburst of Cygnus X-3

FIVE large outbursts of radio emission from Cygnus X-3 have been reported, (refs 1–3 and V. A. Hughes, unpublished). We present here observations of a sixth, which occurred in June and July 1973. The observations were made using the 46-m telescope of the Algonquin Radio Observatory at 10.6 GHz (bandwidth ~100 MHz, system temperature ~120 K). A total of 60 flux density measurements, each providing 7 min of integration time on the source, were made during the period June 25 to July 6, 1973. These observations were taken at sporadic intervals during our programme of measuring early-type emission-line stars, and the observing technique described in ref. 4 was used.

In Table 1 and Fig. 1 we give the measured flux density values for Cygnus X-3. Allowance has been made for the effects of confusion, using data provided by P. C. Gregory which had been derived from observations of the first recorded outburst. The error bars are standard deviations representing the combined effect of random noise (which in many cases was large, due to poor weather conditions), variations in calibration level, and several smaller factors such as the uncertainty due to confusion effects. The flux density scale is based on 3C123 = 7.92 f.u., and 3C274 = 37.1 f.u.

The decay of the sixth outburst was exponential. An exponential decay was also noted for burst 1 (ref. 5), and our analysis of the published data for the other outbursts

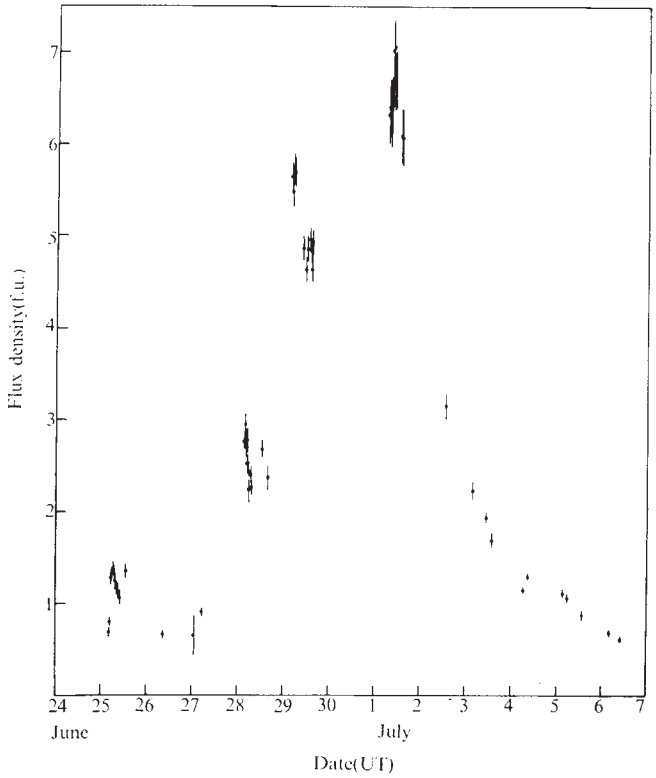


FIG. 1 Measured flux densities of Cygnus X-3 at 10.6 GHz during the sixth outburst.

indicates similar behaviour, suggesting that an exponential decay is a general characteristic of Cygnus X-3 radio bursts. This is clearly incompatible with source models involving simple adiabatic expansion, which predicts a power-law decay[6].

We have developed an alternative model for the Cygnus X-3 radio source. We propose that ambient gas affects the radiation from the synchrotron source in two ways: (i) A high density gas occupies part of the path traversed by the synchrotron-emitting electrons, causing them to lose energy primarily by bremsstrahlung losses. This would explain the exponential decay of the radio bursts, as was suggested[5] for the first outburst. (ii) Each outburst is accompanied by an expanding cloud of ionised gas which surrounds the synchrotron source. If the cloud is initially optically thick at a particular frequency, then the synchrotron source will be obscured. As the gas expands, the decreasing free-free optical depth will result in an increase of the observed intensity. Further the observed outburst will be progressively delayed at lower frequencies. In that case, the form of the rise would depend primarily on the characteristics of the expanding cloud. But if the cloud is optically thin at a particular frequency, then the synchrotron source will be unobscured, and the outburst will be observed simultaneously at all higher frequencies. In that case the form of the rise depends directly on particle injection in the synchrotron source.

This model explains several features of the observed bursts: the exponential decay, the time delay at lower frequencies of some bursts, and the simultaneous rise over a wide range of frequencies of other bursts.

It is interesting that analyses of Cygnus X-3 based on its radio spectrum[7] and X-ray characteristics[8] have also led to models which require the presence of a thermal absorbing cloud.

TABLE 1 Measured flux densities of Cygnus X−3 at 10.6 GHz during the sixth outburst

Date	Time (UT)	Flux density (f. u.)	Standard deviation (f. u.)
June 25	0417	0.678	0.051
	0447	0.799	0.057
	0513	1.284	0.083
	0548	1.330	0.085
	0618	1.389	0.088
	0626	1.339	0.085
	0639	1.246	0.081
	0738	1.172	0.076
	0813	1.135	0.074
	0936	1.059	0.071
	1248	1.359	0.087
June 26	0842	0.671	0.050
June 27	0101	0.65	0.22
	0705	0.911	0.042
June 28	0239	2.764	0.081
	0249	2.96	0.11
	0259	2.815	0.082
	0310	2.709	0.079
	0336	2.77	0.12
	0403	2.527	0.096
	0414	2.45	0.11
	0425	2.24	0.13
	0441	2.424	0.072
	0549	2.263	0.068
	1207	2.691	0.079
	1456	2.38	0.13
June 29	0309	5.65	0.15
	0320	5.48	0.15
	0330	5.73	0.16
	0340	5.70	0.15
	0920	4.87	0.13
	1038	4.63	0.13
	1048	4.86	0.13
	1234	4.96	0.13
	1248	4.84	0.13
	1256	4.64	0.13
	1304	4.93	0.13
July 1	0603	6.32	0.30
	0609	6.40	0.30
	0619	6.38	0.30
	0632	6.27	0.30
	0645	6.44	0.30
	0823	6.70	0.32
	0834	7.02	0.33
	0815	7.06	0.33
	0842	6.66	0.31
	0930	6.70	0.32
	1253	6.09	0.29
	1331	6.07	0.29
July 2	1257	3.15	0.15
July 3	0330	2.23	0.11
	1100	1.942	0.056
	1403	1.686	0.089
July 4	0641	1.153	0.039
	0911	1.295	0.042
July 5	0305	1.118	0.040
	0520	1.055	0.042
	1340	0.866	0.064
July 6	0402	0.692	0.034
	0950	0.612	0.033

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[1] Gregory, P. C., Kronberg, P. P., Seaquist, E. R., Hughes, V. A., Woodsworth, A., Viner, M. R., and Retallack, D., *Nature*, **239**, 440 (1972).
[2] Branson, N. J. B. A., Martin, A. H. M., Pooley, G. G., Readhead, A. C. S., Shakeshaft, J. R., Slingo, A., and Warner, P. J., *Nature phys. Sci.*, **239**, 134 (1972).
[3] Braes, L. L. E., Miley, G. K., Shane, W. W., Baars, J. W. M., and Goss, W. M., *Nature phys. Sci.*, **242**, 66 (1973).
[4] Purton, C. R., Feldman, P. A., and Marsh, K. A., *Nature phys. Sci.*, **245**, 5 (1973).
[5] Aller, H. D., and Dent, W. A., *Nature phys. Sci.*, **239**, 123 (1972).
[6] Van der Laan, H., *Nature*, **211**, 1131 (1966).
[7] Gregory, P. C., and Seaquist, E. R., *Bull. Am. astr. Soc.*, **5**, 394 (1973).
[8] Davidsen, A., and Ostriker, J. P., *Bull. Am. astr. Soc.*, **5**, 411 (1973).

Distribution of late Pleistocene aeolian deposits in eastern and southern England

THERE has been much recent interest in the possible existence of widespread aeolian deposits in Britain. In southern England brickearths at Pegwell Bay[1–3] (TR36) and elsewhere[4] have been interpreted, although without mineralogical confirmation, as extensions of European loess. Silt, in excess of that which could be derived from weathering of the substrata and generally considered loessial, is found in many British soils[5–10] but it is not ubiquitous. For example, although it occurs in soils on Chalky Boulder Clay in Essex[11], on the same till in central East Anglia and on the Chalk of the Breckland and north-west Norfolk it is replaced by sand.

The lithology, and hence likely weathering products, of the Chalky Boulder Clay are very uniform over a large area of eastern England[12]. The till thus provided a unique substratum on which to survey the distribution of sandy and silty surface deposits. The patterns were later traced onto

TABLE 1 Soil provinces in East Anglia and East Midlands

Province		I	II	III	IV	
Type of composition curve		Unimodal	Bimodal	Unimodal	Biomodal	
Peak particle diameters						
Sand grade	Range		120–390	150–370	150–350	
	Mean		201	219	236	
	Standard error		33.5	37.8	55.2	
	Mode		210	210	210	
Silt grade	Range	26–68	16–48		28–50	
	Mean	48.8	31.8		37.2	
	Standard error		0.50		0.75	
	Mode		30		37	
No. of samples		5	141	102	46	Total 294

other formations and into other areas. The survey is summarised here and will be described in detail elsewhere.

East Anglia and east Midlands: The top 4 cm of the soil were sampled at least once in every 10 km grid square containing Chalky Boulder Clay in East Anglia and, at a slightly lower density, in the East Midlands with appropriate replication (190 samples). To minimise effects of solifluxion or downwash each sample was taken from the top of an interfluve, normally the highest point in the square.

Mechanical analysis of the carbonate-free fraction < 2 mm of every sample showed that the soils were unlike any conceivable weathering residue of the under-lying Chalky Boulder Clay and could be grouped into four provinces defined by the unimodal or bimodal form of the composition curves and by peak particle diameters (Fig. 1 and Table 1). Soils of province I are clearly enriched in silt, those of III in sand and those of II and IV in silt and sand. Once recognised on the lithologically uniform till the provinces were traced across interfluves on Cretaceous sands, Chalk, Tertiaries, Norwich Brickearth and the March and Observatory Gravels of Cambridgeshire; and onto flat sites on the Terrace Gravels of the Ouse, Cam and Lark (104 samples). Their distribution is shown in Fig. 2; they transgress geological boundaries, are unrelated to land-form or altitude and, except between I and II, are sharply separated.

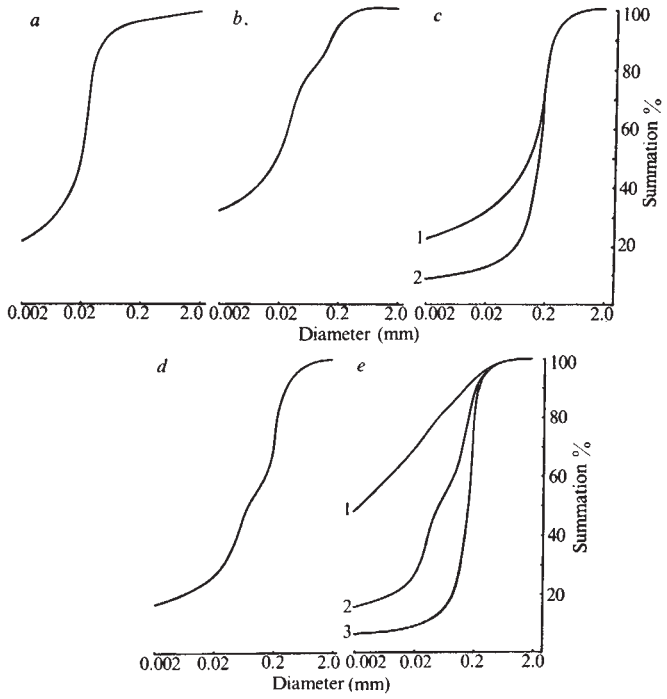


FIG. 1 Cumulative mechanical composition curves. *a*, *b*, Topsoils typical of provinces I and II respectively. *c*, Two topsoils typical of province III; 1, High-clay topsoil overlying and incorporating material from Chalky Boulder Clay; 2, Low-clay topsoil overlying the Chalk. *d*, Topsoil typical of province IV. *e*, Section at Great Blakenham, Suffolk; 1, Chalky Boulder Clay at depth of 4.5 m; 2, Topsoil, showing addition of silt and sand; 3, Sand pocket very low in silt at 1.0 m.

There is a tendency, shown by differences between the means and modes, for peak diameters in the sand grades to decrease southwards, although ranges are similar (Table 1); no east-west trends have been observed. Peak diameters in the silt grades show no consistent geographical relationships.

Heavy mineral counts on the fraction 105 to 63 μm (65 samples) showed that the soils of Province III are the least like the till (Table 2), being significantly different in total heavy minerals ($P < 1.0$) and in opaques, rutile, zircon, epidote, amphiboles and micas. Those of provinces II and IV differ significantly in opaques, zircon and micas, and those of I differ in garnet, rutile, zircon and micas, although only five samples have been examined. Apart from the absence of mica in III there are no qualitative differences between the till and the various soils, suggesting that all the sand grades may have a common ultimate source. Quantitative differences could reflect derivation from different parts of that source or modifications during transport.

Stones in the topsoils cannot be compared directly with those in the till since chalk, the commonest erratic, dissolves in soil formation. But counts of 500 non-calcareous stones at each of 25 sites showed that there are no significant differences between the till and the soils. This suggests, though it does not prove, that surface drifts on the Boulder Clay are not merely the remnant of a later glaciation[12].

The only satisfactory explanation for all these observations is that thin silty and sandy drifts were added to the surface and incorporated by processes such as frost-heaving and biological mixing. Their wide persistence across differing substrata, their invariable occurrence on the highest interfluves and, within each province, the uniformity of peak particle diameters (with values typical of blown sands and loess) support aeolian origins and argue against deposition by water, ice or ablation.

In province III, the peak diameters, low silt contents and absence of micas indicate that the soils are probably derived from blown sand more or less mixed with the substrata (Fig. 1*c*). Since the Breckland falls clearly in this province it now seems probable that the sandy-chalky drift and soils of that area are derived ultimately from blown sand rather than a local sandy till[13].

In provinces I, II and IV, loess is the likely source of the widespread silt since heavy minerals in the fraction 63 to 20 μm are similar throughout. Province IV (46 sites) includes a smaller area (six sites) of 'cover loam' soils reported to contain loess from Devensian deposits[14].

In provinces II and IV the bimodal composition must result from deposition of both sand and silt. Since peak particle diameters in the mineralogically similar sand fractions are comparable in II, III and IV, although with a small trend in mean values, these provinces are probably all parts of one blown sand complex. As pointed out below, the relationship to topography supports this conclusion. The

TABLE 2 Mineralogy of fractions 105–63 μm

Lithology Province	Boulder Clay I, II, III & IV		East Anglia and East Midlands Topsoils I		II		III		IV		Lincolnshire III		Kent IIa
	Mean	s.e.	Mean	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean
Heavy Minerals*	2.54	0.254	1.20	1.50	0.199	1.57	0.160	0.99	0.152	0.75	0.094	1.88
P				<1.0		<1.0		<0.2		<0.2		
Opaques†	85.0	1.08	87	75.2	2.30	72.6	1.94	58.1	3.46	72.2	3.21	86
Rutile group‡	2.9	0.65	10	4.6	0.65	8.1	0.66	5.1	1.20	11.1	1.14	12
Staurolite	3.3	1.08	4	2.7	0.38	2.6	0.29	2.3	0.33	1.8	0.54	5
Tourmaline	5.5	0.84	9	8.9	1.08	9.4	0.97	5.9	0.81	7.6	1.49	6
Garnet group	21.0	1.40	12	26.8	1.33	24.1	1.15	20.2	2.10	17.5	1.87	8
Zircon	9.5	0.92	25	15.5	1.25	36.9	1.88	17.8	2.99	40.6	3.60	47
Epidote group	19.7	1.44	23	19.2	1.17	12.6	0.92	24.3	2.29	7.8	1.31	5
Mica group	12.3	2.24	2	1.1	0.39	0		3.4	2.45	0		0
Amphibole group	23.8	1.78	15	21.0	1.44	6.9	0.56	20.3	2.34	10.4	1.56	11
Others	0.4	0.35	0.2	0.4	0.16	0.1	0.08	0.3	0.13	2.5	0.70	3
No. of samples	33		5	25		29		9		17		3

* Percentage by weight of fraction 105 to 63 μm e.s.d.
P is the measure of significance of the difference of the heavy mineral weight percentage from the Chalky Boulder Clay.
† Percentage by number of total heavy minerals.
‡ Percentage by number of non-opaque heavy minerals.

suggestion that the sand component in the cover loam soils was derived from subjacent deposits[14] may be locally true but is not generally valid as the provinces cross some formations which could not provide such material.

Lincolnshire and Yorkshire: Mechanical analysis of the topsoils from 55 interfluve sites in Lincolnshire and east Yorkshire showed that province III extends beyond the Fens and continues across the Humber. The heavy minerals in these areas and those of III in East Anglia are not significantly different (Table 2). Just as the Breckland seems to be a thick accumulation of the sands of this province in Norfolk, so the 'cover sands' south of Scunthorpe (SE91) appear also to be mechanically and mineralogically part of it rather than a purely local deposit redistributed from the Trias[15].

The boundary between provinces II and III was traced as far as Sleaford (TF04) (Fig. 2). On the east side of III silt accumulations were found on the Lincolnshire and Yorkshire Wolds (Va and Vb). East of the Wolds other Devensian deposits and alluvium make interpretation difficult but there was no evidence for any extension of province IV at any of the fourteen sites examined.



FIG. 2 Distribution of provinces in eastern and southern England. —•—•—, Province boundaries; — — — —, present limits of sampling. Ch, Cheltenham; H, Harwich; Cm, Cambridge; L, Lincoln; Co, Coventry; N, Norwich; Ct, Canterbury; Y, York; L, loess zone in northern France and Belgium; S, cover sand zone in Belgium and the Netherlands.

Midlands and southern England: A further 252 interfluves were sampled, on the Boulder Clay and on other formations suitable for the detection of extraneous material. These were granites, serpentine and gabbro; Devonian, Carboniferous and Jurassic limestones; Jurassic clays, the Lower Greensand, Chalk, Clay-with-flints and various gravels. All areas previously shown to contain loessial soils[5–9] were included. Sampling density was lower than in East Anglia and unsuitable substrata excluded many areas, particularly in southern England (Fig. 3).

Mechanical analysis of all samples showed that the boundary between provinces I and II, although not clearly defined, extends westwards to near Cheltenham (SO92) and south-eastwards into Thanet, where there is a small area of soils (IIa) mechanically similar to those of II but with quantitative differences in mineralogy (Figs 2 and 3, and Table 2).

In province I, although their extraneous origin was sometimes uncertain, accumulations of silt with peak diameters ranging from 16 to 50 μm were found in 177 samples (Fig. 3). The conclusion that this persistent silt is loess is supported by heavy mineral compositions of the fractions 105 to 63 μm and 63 to 20 μm. The former is generally closely similar to the same grade in the substratum but the latter is fairly constant over the whole area (Table 3).

Within province I there are locally a few bimodal soils. Although some of their sand fractions are clearly derived from substrata, such as Lower Greensand or remnants of Reading Beds on Chalk[9], others seem to be definitely aeolian. More intensive sampling might allow this province to be sub-divided.

Peak diameters for the sand fractions of some soils of provinces II, III and IV lie above 210 μm ('coarse' soils), the remainder below ('fine'). The frequency of occurrence of 'fine' soils is unrelated to province boundaries or geographical coordinates but shows a statistically significant tendency to increase with altitude (Table 4).

In East Anglia the long axis of the area of high altitude 'fine' soils lies perpendicular to the boundary between II and III suggesting that these contain the same blown sand complex which has been similarly affected by topography in both of them.

Since there is no relationship between silt grade peak diameter and either geographical position or altitude, it is possible that, whereas during saltation finer sand could better surmount gradients than coarse, silt deposited from suspension was more influenced by local factors such as air turbulence or vegetation.

In East Anglia, the sands of provinces II, III and IV are frequently involuted into the Boulder Clay, presumably by

TABLE 3 Heavy minerals in soils from southern England and northern France

Locality	Seworgan Cornwall SW7131		Brixham Devon SX9456	Cannington Somerset ST2440	Wellow Somerset ST7459	Chalton Hants SU7315	Peter's Green Herts TL1419		Cliffsend Kent TR3564		Hucqueliers France	Forêt d'Eperlecques France
Substratum	Granite		M. Devonian Limestone	Carboniferous Limestone (d_2)	Gt Oolite Limestone	U. Chalk	Chalky Boulder Clay		Brickearth		Clay-with-flints over Chalk	Clay-with-flints over Chalk
Opaques*	27	58	100 72	51 35	100 56	100 61	84	58	86	60	100 56	100 63
Rutile group†		<1	7	11	8	4	10	8	2	10	8	10
Staurolite		4	<1			<1	2	<1	7	1		<1
Tourmaline		1	<1	1	<1	1	5	<1		1	<1	1
Garnet group		4	8	6	10	4		5	7	7	10	42
Zircon		17	24	25	17	19	49	27	41	19	17	7
Epidote group		18	44	43	50	39	12	42	26	39	50	20
Mica group	99‡	50‡	<1	2	7	2	2	2	2	2	<1	1
Amphibole group		17	15	11	7	30	20	14	13	30	14	19
Others	1	3	<1	1	<1	<1		<1	2	<1	<1	1
Barite				100§ 30§								

* Percentage by number of total heavy minerals
† Percentage by number of non-opaque heavy minerals
‡ Note the specially high proportion of micas in both fractions of granite soils
§ Barite is locally present as veins in the limestone in this area
The first column under each locality is for the fraction 105 to 63 μm, the second for the fraction 63 to 20 μm.

cryoturbation. Sections along the margin of the Breckland show that this was contemporary with that producing the well-known patterned ground[13] which, being closely related to present topography, is generally regarded as Devensian. The sands must thus be at least as old as the last major periglaciation in the area. But topsoils typical of Provinces II and III have been traced onto the March and Observatory Gravels and all three Terrace Gravels of the Ouse, Cam and Lark. The youngest of these, the First Terrace[16], has been dated at 19,500 ± 650 BP (ref. 17) suggesting a probable maximum age for the sand.

In contrast to the sands, no interfluve sites are known in East Anglia at which the silt has suffered periglacial disturbance and at Great Blakenham, Suffolk (TM103503) it is clearly younger than the sand: the soil here is bimodal but with increasing depth into the weathered till it becomes more sandy and there are deep sand pockets devoid of silt (Fig. 1*e*). On may deduce that the sand arrived first, was incorporated by cryoturbation and then covered by silt which was mixed by biological agencies to give the present sandy-silty topsoil. This is the most likely origin of all the bimodal soils for it is difficult to envisage any mechanism by which sand and silt could arrive simultaneously. Explanations for the distribution of provinces and the sharpness of most boundaries can so far be only speculative.

There is further evidence that the interfluve silts in the south-east are relatively late. In the present study they have been traced by mechanical and mineralogical analysis across interfluves in Kent into the top of the brickearth at Pegwell Bay which lies above the highest frost structures[1,2]. The survival of silt on exposed scarp crests on the South Downs and Yorkshire Wolds also suggests at least some deposition after intense solifluxion ceased[5]. No valid correlations have yet been made between the interfluve silts and valley deposits in southern England reported as loessial[4,18,19].

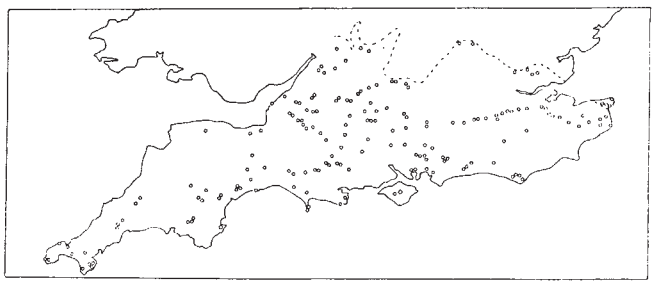


FIG. 3 Soil sampling sites defining province I, and province IIa in the Isle of Thanet. Each circle represents the site of one or more samples. (In the complex area east of Cheltenham sites are too numerous to show individually).

TABLE 4 Peak particle diameter in relation to altitude

Altitude Feet OD	No. of samples 'Coarse' type	'Fine' type	*P*
0–300 (0–90 m)	162	117	<0.1
300–600 (90–180 m)	28	56	<0.1
600–900 (180–270 m)	1	13	<0.1

So it seems that the sands of provinces II, III and IV originally arrived before or during the last intense periglaciation in the Devensian and the silts of I, II and IV later. It is difficult to accept that the latter could belong to the penultimate glaciation as has been suggested for some of the valley brickearths[4].

Provinces I and III show some geographical relation to the zones of loess and cover sands established in Europe[20,21] (Fig. 2). Preliminary mechanical analyses and heavy mineral counts confirm continuity between the silts of province I and loess on the Chalk of northern France (Table 3). But because of the general thinness and reworking of the sands of provinces II, III and IV, no valid stratigraphical correlation can yet be made with the thick series of cover sands in Holland and Belgium. This is being studied further.

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1 Pitcher, W. S., Shearman, D. J., and Pugh, D. C., *Geol. Mag.*, **91**, 308–314 (1954).
2 Kerney, M. P., *Proc. Geol. Ass.*, **76**, 269–274 (1965).
3 Weir, A. H., Catt, J. A., and Madgett, P. A., *Geoderma*, **5**, 131–149 (1971).
4 Tilley, P. D., *Rept. INQUA 6th Warsaw*, **4**, 591–596 (1961).
5 Perrin, R. M. S., *Nature*, **178**, 31–32 (1956).
6 Coombe, D. E., and Frost, L. C., *J. Ecol.*, **44**, 605–614 (1956).
7 Avery, B. W., Stephen, I., Brown, G., and Yaalon, D. H., *J. Soil Sci.*, **10**, 177–195 (1959).
8 Findlay, D. C., *Soils of the Mendip District of Somerset* (Mem. Soil Survey, Harpenden, 1965).
9 Hodgson, J. M., Catt, J. A., and Weir, A. H., *J. Soil Sci.*, **18**, 85–102 (1967).
10 Pigott, C. D., *J. Ecol.*, **50**, 145–156 (1962).
11 Thomasson, A. J., *Soils of Writtle Institute Farm* (Soil Survey of Great Britain, Harpenden, 1960).
12 Perrin, R. M. S., Davies, H., and Fysh, M. D., *Nature phys. Sci.*, **245**, 101–104 (1973).
13 Watt, A. S., Perrin, R. M. S., and West, R. G., *J. Ecol.*, **54**, 239–258 (1966).
14 Catt, J. A., Corbett, W. M., Hodge, C. A. H., Madgett, P. A., Tatler, W., and Weir, A. H., *J Soil Sci.*, **22**, 444–452 (1971).
15 Straw, A., *Trans. Lincs. Nat. Un.*, **15**, 260–269 (1963).
16 Worssam, B. C., and Taylor, J. H., *Geology of the Country around Cambridge* (HMSO, London, 1969).
17 Godwin, H., and Willis, E. H., *Radiocarbon*, **6**, 116 (1964).
18 Mothershead, D. N., *Fld. Stud.*, **3**, 433–453 (1971).
19 Evans, J. G., *Wilts. archaeol. nat. Hist. Mag.*, **63**, 12–26 (1968).
20 van der Hammen, T., Maarleveld, G. C., Vogel, J. C., and Zagwijn, W. H., *Geol. Mijnb.*, **46**, 79–95 (1967).
21 *Excursion guide, Belgium* (edit. by Paepe, R.) (Quaternary Research Association, Brussels, 1970).

Geophysical evidence for the intersection of the St Paul, Cape Palmas and Grand Cess fracture zones with the continental margin of Liberia, West Africa

PUBLISHED reconstructions of Gondwana continent[1] (Fig. 1*a*) show a gap in fit near the junction of the Americas and Africa. To study this critical area, the Unitedgeo I made geophysical measurements and collected rock samples across the continental margin of Liberia (USGS-IDOE cruise leg 5) in November 1971. Figure 1*b* indicates the location of the 5,400 km of ship track on a generalised bathymetric map[2]. We shall discuss the data in detail elsewhere. Here we present the evidence for the existence of three fracture zones, two of which have not been reported previously, intersecting the continental margin at the north end of the South Atlantic, which remained closed probably until Cretaceous time. We suggest that Precambrian structures on the African continent controlled the location of these fracture zones. Figure 1*c* compares gravity and magnetic profiles and interpretations of the seismic profiles for three selected lines (27, 30 and 34) crossing the Grand Cess, Cape Palmas and St Paul fracture zones, respectively.

The bathymetric data (Fig. 1*b*) indicate that the north-west section of the continental margin of Liberia is narrow, the slope is moderately rough and the average gradient is about 3° (ref. 2). South-eastwards to about 9°30′W (about line 22) the slope gradually becomes smooth and wider and the average gradient is about 1.8°, which suggests depositional outbuilding. The topography of the area southeast of about line 22 reflects the effects of the inferred three fracture zones (the Grand Cess, Cape Palmas and St Paul fracture zones), where locations as indicated in Fig. 1*b* are interpreted from the magnetic and gravity data. Figures 2 and 3 show the magnetic and gravity profiles normal to the coast and indicate the anomaly correlations over the inferred fracture zones. The anomalies on lines 1 to 21, north-west of the fracture zones, are not discussed here but are shown for comparison. The St Paul fracture zone has been reported previously in this area by others[4–6]. The comparison profiles shown for lines 27, 30 and 34 (Fig. 1*c*) are typical of the other lines crossed by the inferred fracture zones as indicated in Fig. 1*b*.

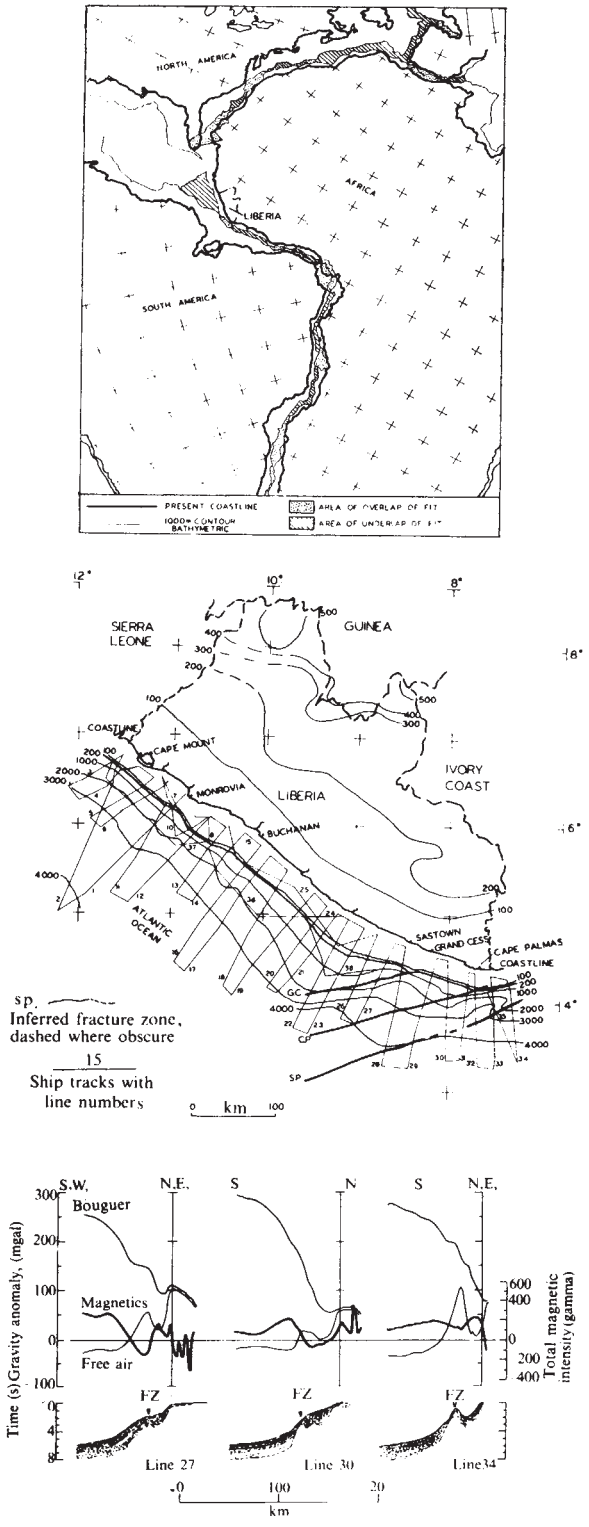


FIG. 1 *a*, Reconstruction showing predrift configuration of parts of continents around the Atlantic Ocean, taken from ref. 1. Note the good fit in the eastern third of Liberia and the poorer fit in the western two-thirds. Contours in metres. *b*, Index map showing generalised bathymetric and surface-elevation contours, as well as ship-track lines. Locations of Grand Cess, Cape Palmas, and St Paul fracture zones are indicated, as inferred from magnetic and gravity data. *c*, Geophysical profiles along lines 27, 30 and 34 crossing the Grand Cess, Cape Palmas and St Paul fracture zones, respectively. Seismic reflection interpretations are shown at bottom in two-way time.

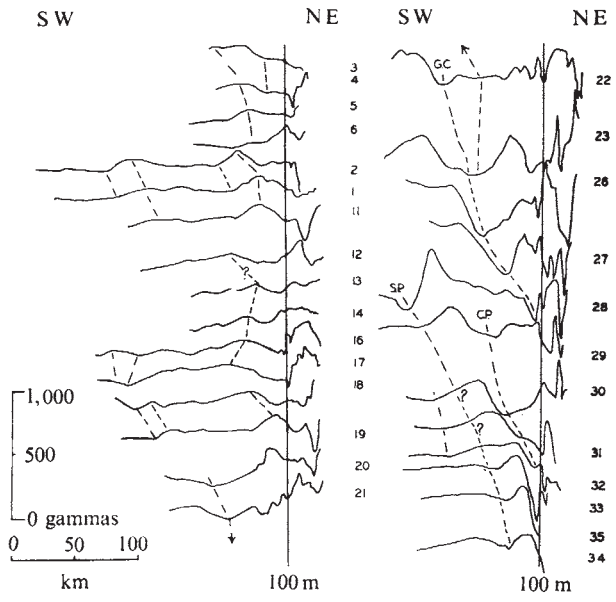


FIG. 2 Magnetic profiles for indicated track lines approximately normal to coast with IGRF removed. Suggested anomaly correlations are indicated. Tics indicate the inferred Grand Cess (G.C.), Cape Palmas (C.P.) and St Paul (S.P.) fracture zones are based on magnetic and gravity data.

We interpret the features labelled as the Cape Palmas and Grand Cess fracture zones on the basis of their similarity in geophysical character to the St Paul fracture zone. The extension of the Cape Palmas and St Paul fracture zones south-west of our ship tracks is based on earlier published magnetic data[4].

Behrendt and Wotorson[7,8] presented data over the land and continental shelf and compiled a tectonic map based on these data and available geological information. Rock foliation and tectonic features trend east-northeastwards in the Eburnean province of south-east Liberia, as illustrated by aeromagnetic data (Fig. 2). These linear anomalies are associated with isoclinally folded and faulted paragneisses of Eburnean age (~ 2,000 m.y.). Comparison of the trends of the fracture zones (Fig. 1*b*) with the trends of the magnetic anomalies of Fig. 4 shows that they are similar. The marine magnetic anomalies over the Grand Cess and Cape Palmas fracture zones (Fig. 1*c*) are continuous into the aeromagnetic anomalies of Fig. 2, although they are of longer wavelength because of greater water depths and depth of magnetic basement on the marine data. The magnetic anomaly associated with the St Paul fracture zone passes out of the surveyed area; this fracture zone intersects the margin of West Africa farther east[4–6] (Fig. 1*b*). We do not imply, from this correlation, that the fracture zones and sources of magnetic anomalies on the continent are the same; the great discordance in ages between the Eburnean province (about 2,000 m.y.) and the time of rifting of Africa and South America during the Cretaceous precludes that. We interpret this coincidence in trends to indicate that the isoclinal folds and faults in the Eburnean province might have provided structural control for the location of the fracture zones developed at the time of rifting. In coastal Liberia the geological evidence of actual faulting that could definitely be said to be syntectonic with rifting is lacking, although work by Francheteau and Le Pichon[5] suggests this possibility. A fault inferred from aeromagnetic data and confirmed by geologic mapping on land (A. H. Chidester, personal communication) occurs in the area where the Grand Cess fracture zone intersects the shelf. The tectonic map of Liberia[8] also reveals several structures where the Grand Cess fracture zone intersects the shelf. We do not know the age of these features but they could be related to the fracture zones.

Though now buried, as indicated from the seismic data of Fig. 1*c*, the three fracture zones reported here have influenced subsequent deposition of sediments and the physiography of the slope and rise. Probably the broadening of the slope between line 13 and 22 (Fig. 1*b*) is related to the fracture zones, in the manner suggested by Francheteau and Le Pichon[7] for the fracture zones in the Gulf of Guinea. This area, northwest of line 22, is the area of the gap in the Bullard *et al.*[1] reconstruction and was probably part of the continental margin earlier (at the time of the opening of the North Atlantic) than the area crossed by lines 23 to 35 (which originated at the time of the South Atlantic opening). At the scale of the entire South Atlantic, these fractures zones are very close together; in fact, they all lie easily between the 17° and 19° flow lines about Francheteau and Le Pichon's[7] early pole of opening. The continuation of these inferred fracture zones into the similar northeast trending magnetic anomalies of the south-east Liberian continental shelf (Fig. 2) led us to conclude that the Precambrian isoclinally folded and faulted metamorphic terrane influenced their orientation and location at the time of opening of the South Atlantic.

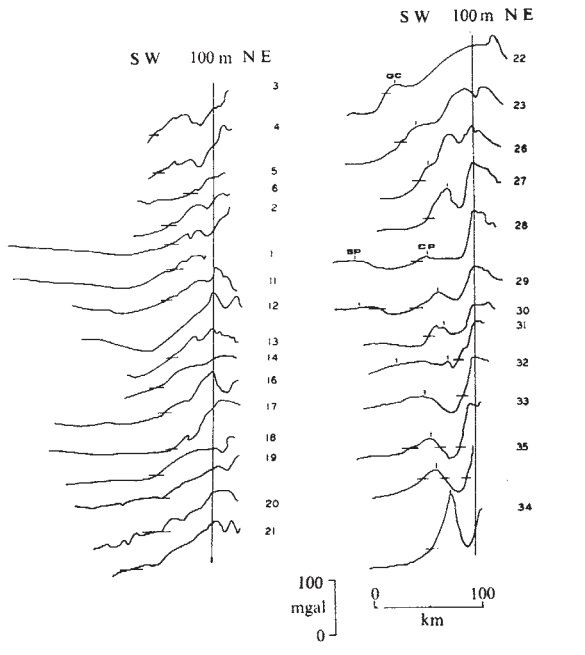


FIG. 3 Free air anomaly profiles for indicated track lines approximately normal to coast. Vertical bars indicate locations of Grand Cess (G.C.), Cape Palmas (C.P.) and St Paul (S.P.) fracture zones as inferred from magnetic and gravity data; horizontal bars indicate 0 mgal.

Cochran[9] showed several approximately north trending gravity and magnetic profiles west of our data (from about 15°W to 43°W). He identified the St Paul fracture zone crossing the Atlantic to the South American margin on the basis of its characteristic appearance on these profiles. The profiles on the African side (V2713 and V2604, in particular) and the profile nearest South America (V2502) are similar to those presented here. Just north of the anomaly associated with St Paul fracture zone on Cochran's data, are features we identify as associated with the Cape Palmas and Grand Cess fracture zones as defined here.

Probably the St Paul, Cape Palmas, and Grand Cess fracture zones are Cretaceous in age, at the intersection of the African continental margin, based on available information on the date of separation of Africa and South America (about 140 m.y. ago[6]). Previous interpretations[7,8] that North America separated from Africa about 176 to 192 m.y. ago northwest of about line 22 (Fig. 1*b*) would require that the oceanic basement in this area be about 40 m.y. older than that to the

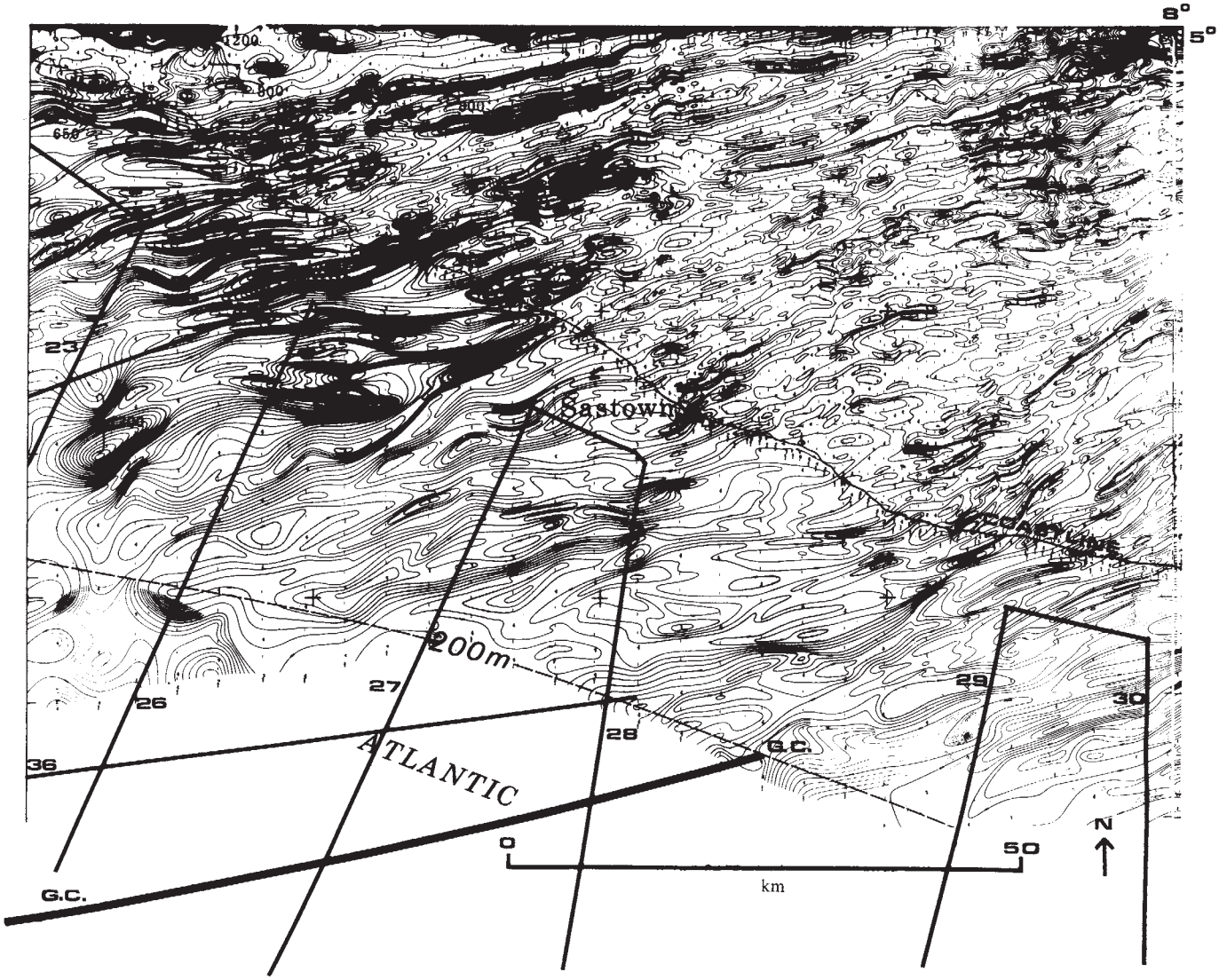


FIG. 4 Portion of aeromagnetic contour map in Sastown area with ship-track lines indicated. Contour interval is 10 and 50 gamma. Inferred location of Grand Cess fracture zone is indicated (G.C.). High amplitude anomalies are indicated in gammas.

south-east of line 22. If the Deep Sea Drilling Project (phase 3 leg 40) were to locate several holes in this area it would test this interpretation.

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1 Bullard, E. C., Everett, J. E., and Smith, A. G., *R. Soc. Lond. Phil. Trans.*, **A258**, 1088 (1965).
2 Robb, J. M., Schlee, J., and Behrendt, J. C., *US Geol. Surv. J. Res.*, **1**, 5 (1973).
3 Schlee, J., *US Dept Commerce, natn. tech. Inf. Service*, PB209-871 (1972).
4 Arens, G., Delteil, J. R., Valery, P., Damotte, B., Montadert, L., and Patriat, P., *Great Britain Inst. geol. Sci. Rept*, 70/16 (1971).
5 Francheteau, J., and Le Pichon, X., *Am. Ass. Petrol. Geol. Bull.*, **56**, 6 (1972).
6 Le Pichon, X., and Hayes, D. E., *J. geophys. Res.*, **76**, 26 (1971).
7 Behrendt, J. C., and Wotorson, C. S., *Geol. Soc. Am. Bull.*, **81**, 12 (1970).
8 Behrendt, J. C., and Wotorson, C. S., *US Geol. Surv. Prof. Pap.*, 810 (in the press).
9 Cochran, J. R., *Geol. Soc. Am. Bull.*, **84**, 10 (1973).

Production of copper(I) halide emission spectra by MECA

THE introduction of copper(II) salts into most commonly used flames gives a green emission. Copper halides give a particularly intense emission, which is used in the well-known Beilstein test for halogens. The spectra from all copper salts, however, are very similar, and consist mainly of diffuse CuOH bands in the region 535–555 nm, a CuH band centred around 429 nm, CuO emission bands between 445 and 492 nm and a copper atom line at 327 nm (ref. 1, and Fig. 1). Copper(I) halide bands are also present when copper(II) halides are used, but are weak. The enhanced emission of the halides, therefore, arises mainly from their great volatility and consequent enhanced production of exited CuOH, CuO and CuH molecules, rather than greatly enhanced production of copper(I) halide emission. For this reason, pseudohalides such as cyanide and thiocyanate ions also give positive

Beilstein tests. The copper(I) chloride emission that has been observed in flames, especially when they are very fuel lean[2], is very insensitive for use in analysis.

Recently, we have developed a new, highly sensitive analytical flame technique named molecular emission cavity analysis (MECA)[3]. In this technique, the sample is placed in a cavity at the end of a steel rod, and the cavity is positioned in a hydrogen diffusion flame so that the flame gases flow almost vertically past the entrance to the cavity, with a small proportion entering the cavity. The flame also heats the exterior of the cavity. The emission characteristics of many materials placed in the cavity are appreciably different from those obtained by aspirating the sample solution into the flame. When microgram amounts of copper(II) chloride are placed in the cavity, or when traces of hydrochloric acid are introduced into a copper-plated cavity, the emission spectrum is very different from that obtained by aspirating an aqueous copper chloride solution into the flame. The spectra are compared in Fig. 1. Particularly noticeable is the much reduced intensity of the CuH, CuOH and CuO bands, and the greatly enhanced bands which can be attributed to copper(I) chloride emissions. The wavelengths of the main emission peaks are summarised in Table 1. The apparent triplet at the lowest wavelengths corresponds closely, in wavelength and relative intensity, with the main peaks of the D and E systems[1] of copper(I) chloride. Similarly the middle triplet corresponds with the B and C systems, and the longest wavelength triplet with the A system.

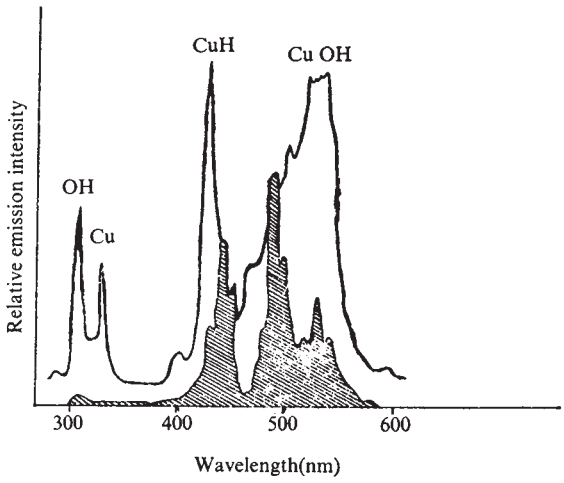


FIG. 1 Spectra of $CuCl_2$ in aqueous solution aspirated into H_2-N_2 diffusion flame (line drawing) and in the cavity (MECA) in the same flame (solid drawing).

Copper(II) bromide and iodide give significantly different spectra in the cavity. The wavelengths of the main peaks are summarised in Table 1. The copper bromide peaks at 480 nm and above correspond closely to those of the A system of copper(I) bromide, whereas the copper iodide peaks at 509 nm and above correlate well with those of the A system of copper(I) iodide. In all instances, the peaks at 437 nm do not correspond to CuH emissions, which would seem to be the only other possible emitters in this wavelength range.

The emissions can be used to detect nonogram amounts of halides. Other copper salts, such as copper(II) sulphate and nitrate, give no emission in the cavity.

Small amounts of tin(II) chloride or bromide in the cavity produce broad emission bands, with peaks at 440 and 460 nm, and 460, 480, 490 and 495 nm respectively. These do not correspond to the well characterised red SnH and blue SnO emissions but appear to be similar, however, to the blue or blue-green emission that appears just at the base of a hydrogen diffusion flame when the tin halide is aspirated, and is therefore probably tin halide emission. No spectral measurements of tin halide emissions in flames, however, have been reported. Similar emissions are obtained from halides of indium, lead, cobalt, cadmium and germanium.

TABLE 1 Wavelengths (nm) of major peaks in MECA spectra

CuCl	CuBr	CuI
425 (4)	437	437
437 (8)		
442 (5)		
475 (4)		
488 (10)*		
497 (6)		
515 (3)	480	509–511*
527 (5)	488*	514
539 (3)	496	520
	503	531

* most intense peak.
(relative intensities in parenthesis)

The reason for enhanced halide emission from the cavity has not been completely established. It would seem to result, however, from the limited radical concentration in the flame gases within the cavity, which reduce the formation of non-halide copper species, and the relatively cool cavity surface, which enhances the emission of the emitting species as a result of the Salet phenomenon[4].

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1 Pearse, R. W. B., and Gaydon, A. G., *The Identification of Molecular Spectra* (Chapman and Hall, London, 1963).
2 Honma, M., *Analyt. Chem.*, **27**, 1656 (1955).
3 Belcher, R., Bogdanski, S. L., and Townshend, A., *Analytica chim. Acta*, **67**, 1 (1973).
4 Salet, G., *C.r. hebd. Séanc. Acad. Sci., Paris* **68**, 404 (1869); **73**, 559 (1871); **74**, 865 (1872).

Teardrop shapes for small bubbles and drops moving through Newtonian liquids

IN each of the previous observations[1–11] of bubbles and drops with pointed tails the continuous liquid has had non-Newtonian characteristics. For Newtonian liquids, the shapes of drops and bubbles have been generally reported to pass smoothly from spherical to oblate ellipsoidal with increasing volume. Here we report observations of teardrop shapes for bubbles and drops in Newtonian liquids over a narrow range of sizes.

The experiments were done in a plexiglass column of cross section 39 × 32 cm and height 117 cm. The continuous liquid was an aqueous sucrose solution of density 1.393 g cm^{-3} and shear viscosity 12.97 poise. The temperature for all the experiments was 29.5° C. Drops of carbon tetrachloride were injected by pouring from the top surface. Drops of silicone oil and air bubbles were formed under pressure at an orifice at the base of the column. The properties of each of the three systems are given in Table 1. No special precautions were taken to eliminate surface-active contaminants

TABLE 1 Physical properties of the systems

Dispersed fluid	Density of dispersed phase ρ_d (g cm^{-3})	Viscosity of dispersed phase μ_d (poise)	Interfacial tension σ (dyne cm^{-1})	Approximate range of sphere-equivalent diameters over which pointed tails have been observed (cm)
Carbon tetrachloride	1.585	0.0105	34.3	1.13–1.70
Silicone oil	0.92	0.545	53.5	0.68–1.19
Air	0.00126	0.00018	73.0	0.58–0.98

from the liquids studied. In order to characterise the rheological behaviour of the aqueous sugar solution, an extensive series of tests was done with a rheometric mechanical spectrometer and with a Weissenberg Rheogoniometer (model RD-18). No dependence of viscosity on either rate of strain or on time could be observed so long as precautions were taken to ensure constancy of temperature and moisture content. Thus the continuous fluid can be taken to be Newtonian. The dispersed fluids (see Table 1) were also Newtonian.

Tracings of drops and bubbles with a teardrop shape, taken from still photographs, are presented in Fig. 1. Tails are observed only for a limited range of drop and bubble sizes as shown in Table 1. As the drop or bubble size is increased beyond the teardrop range, the tail disappears and is replaced by an indentation or dimple at the rear.

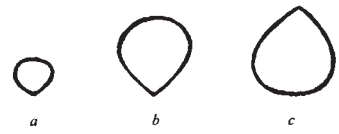


FIG. 1 Shapes of air bubbles, and drops of silicone oil and carbon tetrachloride moving through 70%, w/w, aqueous sucrose solution. *a*, air bubble; sphere equivalent diameter: 0.73 cm; velocity 3.08 s^{-1}. *b*, Silicone oil; 1.19 cm; 2.82 cm s^{-1}. *c*, Carbon tetrachloride; 1.56 cm; 1.80 cm s^{-1}.

Drops or bubbles which were given a teardrop shape by the injection process (for example, if the tail was stretched as a drop pulled away from the injection orifice) or by coalescence tended to retain the teardrop shape whereas those which began as spheres tended to remain so unless they coalesced with other drops or bubbles. In other words, a tail once formed was not retracted but spherical fluid particles in free rise or fall did not tend to form tails on their own. The tails observed were generally less pronounced than those reported by other workers for non-Newtonian liquids. Recently, tailing of a filament of the dispersed liquid has been observed for cases where the continuous fluid is significantly viscoelastic[11]. No such phenomenon has been observed with the Newtonian liquids used in the present work.

We conclude that teardrop shapes are not restricted to non-Newtonian fluids as has been generally believed. On the other hand, the range over which pointed tails can be observed in Newtonian liquids seems to be small and there is little doubt that thixotropic and viscoelastic continuous liquids lead to more pronounced tails and to phenomena, such as trailing filaments, which do not occur for Newtonian liquids.

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1 Arnold, H. D., *Phil. Mag.*, **22**, 755 (1911).
2 Astarita, G., and Apuzzo, G., *A. I. Ch. E. Jl.*, **11**, 815 (1965).
3 Calderbank, P. H., Johnson, D. S. L., and Loudon, J., *Chem. Engng. Sci.*, **25**, 209 (1970).
4 Fararoui, A. and Kintner, R. C., *Trans. Soc. Rheol.*, **5**, 369 (1961).
5 Garner, F. H., Mathur, K. D., and Jenson, V. G., *Nature*, **180**, 331 (1957).
6 Mahtre, M. V., and Kintner, R. C., *Ind. Engng. Chem.*, **51**, 865 (1959).
7 Marrucci, G., Apuzzo, G., and Astarita, G., *A. I. Ch. E. Jl.*, **16**, 538 (1970).
8 Philipoff, *W., Rubb. Chem. Technol.*, **10**, 76 (1937).
9 Savic, P., *Nat. Res. Council Can. Rep.*, NRC-MT-22 (1953).
10 Warshay, M. E., Bogusz, E., Johnson, M., and Kintner, R. C., *Can. J. chem. Engng.*, **37**, 29 (1959).
11 Wilkinson, W. L., *Nature phys. Sci.*, **24**, 44 (1972).

Colonisation of the early ocean floor

THE earliest well documented evidence of macroscopic life is provided by stromatolitic algae from sediments deposited in intertidal or shallow marine environments at least 2,700 m.y. ago[1]. It is generally agreed that the earliest metazoan animals were soft-bodied forms which are rarely preserved but have left tracks, trails and burrows, collectively known as trace fossils. The earliest reliably identified trace fossil is a burrow system considered to have been produced by a worm-like organism, probably an annelid, found in shallow marine clastic sediments of the Grand Canyon Series (USA) suggested to be over 1,000 m.y. old[2]. Vertical burrows have been found in the Buckingham Sandstone in North Australia, also a shallow water deposit dated radiometrically as more than 790 m.y. old[2], and in the Areyonga Formation, of similar age[3]. A more extensive collection of trace fossils has recently been recorded by Webby[4] from the clastic shallow marine late Precambrian (about 600 m.y. old) Torrawangee Group of New South Wales (Australia) with three trace fossil genera positively identified: *Planolites*, *Cochlichnus* and *Torrawanga*. The late Precambrian Ediacara Beds of South Australia, also interpreted as shallow water clastic deposits, have yielded one positively identified trace fossil genus (*Cochlichnus*) and five other forms, all thought to have been produced by metazoa[2]. The common factor in these occurrences is the shallow water environment. Most of the trace fossils are burrows of infaunal deposit feeders which inhabited offshore environments of relatively quiet water; but the record of vertical burrows, now most commonly found in the high energy intertidal zone, may indicate occupation of even the shallowest water niches. There is as yet no indication of colonisation of the deep oceans during Precambrian times.

Tracing the dispersal of even soft-bodied benthonic animals from their initial shallow water habitat into deep water is helped by the facies dependence of the trace fossils which they produce. Seilacher[5] suggested that many trace fossil genera can be assigned to one of three depth-controlled communities and this has, in general, been confirmed by later studies[6–8]. The communities, each named after a characteristic trace fossil are, in order of increasing water depth: *Cruziana* (littoral to wave base), *Zoophycos* (wave base to

TABLE 1 First appearance of ichnogenera

	Cruziana/Zoophycos community (shallow)	*Nereites* community (deep)	Ichnogenera independent of depth
Tertiary	0	5	0
Cretaceous	0	18	0
Jurassic	1	0	0
Triassic	2	0	1
Permian	2	1	0
Carboniferous	2	1	2
Devonian	0	2	0
Silurian	0	2	0
Ordovician	5	7	7
Cambrian	21	3	5
Precambrian	3	0	1

zone of turbidite deposition) and *Nereites* (deep water turbidite zone). Thus, by determining the oldest known occurrence of trace fossil genera belonging to the different communities, it should be possible to trace, in time, the dispersal of benthonic faunas into deep water, away from the shallow water niche which they occupied in the Precambrian. Also, by considering the stratigraphical range of the depth-controlled trace fossils, it should be possible to estimate the diversity of trace fossils, and hence animals, in each depth zone at different times. For this analysis, a wide, and as far as possible non-selective, coverage is necessary.

I have therefore considered all ichnogenera (except trace fossils produced by boring organisms) mentioned in a recently published comprehensive text on trace fossils[9]. This text includes papers on all systems and from widespread areas, so that all common traces and some rarer ichnogenera were included but old, inadequately described, ichnogenera were avoided. Each of the depth-dependent ichnogenera was then assigned to its community and its range noted from the *Treatise on Invertebrate Paleontology*[10], modified where necessary to take account of more recent investigations. In all 81 ichnogenera were included.

The results are presented in Tables 1 and 2. Table 1 records only the first appearance of ichnogenera in the stratigraphic column. Table 2 is based on the stratigraphic range of ichnogenera and each ichnogenus is counted as present in every system within its stratigraphic range whether or not is has been recorded from them all.

The tables show a clear trend of increasing diversity in deep water, and possibly decreasing diversity in shallow water, with time. Extension of the survey to include all trace fossil genera in the *Treatise of Invertebrate Paleontology* made no significant difference to the trends but the results are not reported here because of the uncertainty in assigning many neglected and poorly described genera to a community. The diversification seen in the younger Precambrian rocks is continued and markedly accelerated in Lower Cambrian times. Abundant and surprisingly diverse trace fossils have been recorded from shallow water Lower Cambrian sediments in many parts of the world[2,11–16] but few forms have been found in deep water deposits. Even simple depth-independent forms are rare in this environment. The contrast between shallow and deep water in the numbers and diversity of trace fossils recorded persists throughout Cambrian times. It cannot be due to any lack of suitable deep water deposits because these are widely distributed and the contrast is clearly seen, for example, in Wales where, despite the great number and variety of traces found in the shallow water sediments, extensive searching in the well exposed deep water turbidite sequences has yielded only a few mostly non-diagnostic burrows[17]. Significant colonisation of the deep ocean floor does not therefore seem to have taken place before the close of the Cambrian period.

TABLE 2 Number of ichnogenera present during different periods

	Cruziana/ Zoophycos community (shallow)	*Nereites* community (deep)	Ichnogenera independent of depth	Total
Tertiary	12	30	9	51
Cretaceous	18	31	11	60
Jurassic	19	12	13	44
Triassic	19	12	13	44
Permian	18	12	12	42
Carboniferous	22	13	14	49
Devonian	21	12	12	45
Silurian	22	9	12	43
Ordovician	24	9	13	46
Cambrian	24	3	6	33
Precambrian	3	0	1	4

The widespread shallow water seas of earliest Ordovician (Arenig) times supported an abundant and varied benthos which produced many trace fossils, particularly trilobite traces and infaunal burrows. The first traces assignable to the *Zoophycos* community appeared, including *Zoophycos* itself, which has been recorded low in the Ordovician sequence in Iraq[5], thus testifying to the colonisation of the intermediate depths. There is also evidence of the first significant colonisation of the deep ocean floor with nine ichnogenera recorded from the *Nereites* community. There is, in addition, an increase in the number of depth-independent ichnogenera from 6 to 13. Data from Upper Palaeozoic and Mesozoic strata suggest the possibility of a further decrease in the number of shallow water forms. The well known abundance of trace fossils in the late Mesozoic and Tertiary flysch successions[18–23] confirms an extensive colonisation of deep water at this late stage, although the very sharp increase in number of ichnogenera present may partly reflect the more detailed study to which these deposits have been subjected. Thus, from the Cambrian to the Tertiary, the available data suggest that the total number of ichnogenera has remained roughly constant, but there has been an increase in the diversity in deep water and possibly a decrease in diversity in shallow water.

The initial colonisation of the deep oceans may have been stimulated by high dispersal pressures in the shallow water niches. Trace fossil abundance and diversity suggest, however, that such pressures reached a high level as early as Lower Cambrian times, yet significant colonisation of the deep oceans was apparently delayed by some 70 m.y., until the Ordovician, when dispersal pressures do not appear to have been much greater. This delay may have been the result of low oxygen concentration in the early deep seas. Most deep water benthos are, however, sediment grazing types; their inability to colonise this environment during the Cambrian may also reflect inadequate supplies of organic detritus within the muds of the early deep ocean floor, together with anaerobic decay brought on by oxygen deficiency in such organic material as may have been present.

No doubt the stratigraphical range of many of the trace fossils used in this analysis will be extended as a result of further work. Nevertheless, the main trends are so clearly shown that it is unlikely that such additions will significantly modify the conclusions which are:

(1) The earliest benthonic metazoa shared a shallow water niche with already existing stromatolitic algae but the algae were restricted to calcareous facies while metazoan trace fossils have so far only been found in clastic sediments.

(2) There was rapid diversification in tracks, trails and burrows in early Cambrian shallow water environments. In contrast, only three genera of deep water trace fossils have so far been recorded from Cambrian sediments and significant colonisation of the deep ocean floor appears to have been delayed by some 70 m.y. until the Ordovician when at least nine ichnogenera were present.

(3) In the late Mesozoic and early Tertiary there appears to have been a sharp increase in abundance and variety of trace fossils produced in deep water but this may partially reflect the more detailed study to which these deposits have been subjected.

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1 Rutten, M. G., *The Origin of Life,* 420 (Elsevier, Amsterdam, 1971).
2 Glaessner, M. F., *Lethaia,* **2,** 369 (1969).
3 Ranford, L. C., Cook, P. J., and Wells, A. T., *Bur. Min. Resour. Aust.,* **86,** 1 (1965).
4 Webby, B. D., *Lethaia,* **3,** 79 (1970).
5 Seilacher, A., in *Approaches to Paleoecology* (edit. by Imbrie, J., and Newell, N.), 296 (Wiley, New York, 1964).
6 Crimes, T. P., in *Trace Fossils* (edit. by Crimes, T. P., and Harper, J. C.), **3,** 101 (Seel House Press, Liverpool, 1970).
7 Ager, D. V., and Wallace, P., in *Trace Fossils* (edit. by Crimes, T. P., and Harper, J. C.), **3,** 1 (Seel House Press, Liverpool, 1970).
8 Chamberlain, C. K., *Am. Ass. Petrol. Geol.,* **55,** 34 (1971).
9 *Trace Fossils,* (edit. by Crimes, T. P., and Harper, J. C.), 3, (Seel House Press, Liverpool, 1970).
10 Hantzschel, W., in *Treatise on Invertebrate Paleontology,* (edit. by More, R. C.), 177 (University of Kansas Press, Lawrence, 1962).
11 Seilacher, A., *Neues Jb. Geol. Palaeontol.,* **103,** 155 (1956).
12 Seilacher, A., *Akad. Wissensch. Lit. Abh. Math. – Naturw. Kl. Jahrg.,* **10,** 373 (1955).
13 Banks, N., in *Trace Fossils* (edit. by Crimes, T. P., and Harper, J. C.), **3,** 19 (Seel House Press, Liverpool, 1970).
14 Cowie, J. W., and Spencer, A. M., in *Trace Fossils* (edit. by Crimes, T. P., and Harper, J. C.), **3,** 91 Seel House Press, Liverpool, 1970).
15 Young, F. G., *Can. J. Earth Sci.,* **9,** 1 (1972).
16 Germs, G. J. B., *J. Palaeont.,* **46,** 864 (1972).
17 Crimes, T. P., *Palaeogeogr. Palaeoclimatol., Palaeoecol.,* **7,** 113 (1970).
18 Gomez de Llarena, J., *Monografias Inst. "Lucas Mallada" Invest. Geol.,* **13,** 1 (1954).
19 Sacco, F., *Atti Soc. ital. Sci. nat.,* **31,** 151 (1888).
20 Vassoeivitch, N. B., *Uslowia Obrazowania Flisza,* (1951).
21 Ksiazkiewicz, M., in *Trace Fossils* (edit. by Crimes, T. P. and Harper, J. C.), 3, 283 (Seel House Press, Liverpool, 1970).
22 Tanaka, K., *Geol. Surv. Japan,* **242** (1971).
23 Crimes, T. P., *Sedimentology,* **20** 105 (1973).

BIOLOGICAL SCIENCES

mRNA is expected to form stable secondary structures

Most sequenced RNAs have the potential to form secondary structures by means of local base pairing and loop formation. Arguments have been advanced that these structures are the result of evolutionary pressure, or of requirements for unique functions such as virus packaging[1–7]. To determine whether secondary structure is an intrinsic or anomalous property of RNA we have investigated random sequences generated by computer. These sequences, which contain no real biological information, can be arranged into thermodynamically stable secondary structures with about 50% base pairing. The results imply that one should expect a high degree of base pairing in RNA, consistent with early estimates of helical content in random copolynucleotides[8] and the constraints accompanying structure formation in a hypothetical tRNA molecule[9,10]. Therefore, the mere existence of secondary structure does not necessarily implicate special biological functions, although the potential for functional interactions clearly exists.

Table 1 Pairing percentages in random structures

Sequence length	No. investigated	% of bases paired
37–62	23	42
77	25	55
144	2	52

Table 1 shows the average percentage of bases paired in fifty random sequences of various lengths. A sequence was generated by assigning equal *a priori* probabilities to each base and using a standard random number routine to decide which base occupied a particular position along the chain. In each case the computer printed out the base pairing matrix[11], facilitating the choice of pairing arrangements by inspection. These arrangements were then evaluated according to rules detailing thermodynamic stability[11–15], and the most stable secondary structure was selected in each of the fifty cases. It is possible that occasionally even more stable structures were overlooked; this implies that the resulting base pairing percentages (Table 1) are minimum values. Nevertheless, the most stable structures on the average have 40–60% of bases paired (an example of a random sequence with about 60% pairing is shown in Fig. 1).

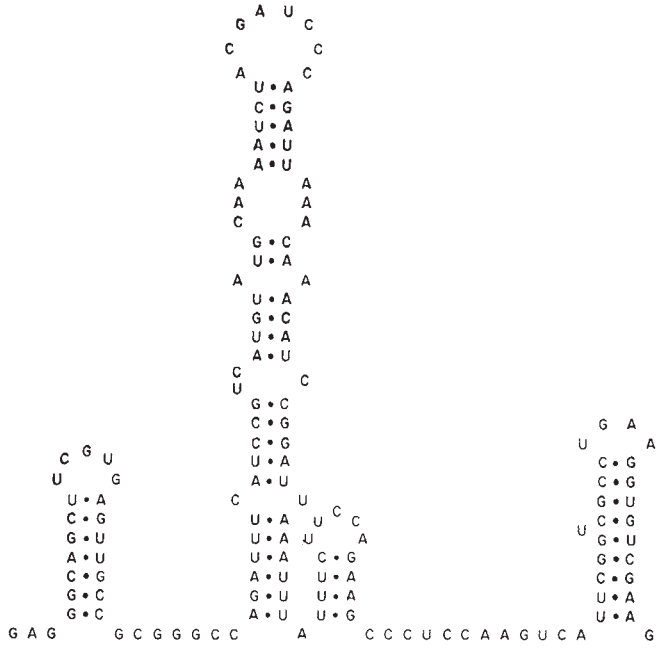


Fig. 1 One possible secondary structure for a random sequence of 140 nucleotides. Approximately 60% of the bases are paired.

At fixed length the extent of base pairing observed is not highly variable. Fig. 2 shows the distribution of base pairing in twenty-five random sequences of seventy-seven nucleotides each. Fully 80% of the most stable structures contain 21 ± 3 base pairs, and no structure includes fewer than 15 or more than 28 base pairs. Even though the number of sequences evaluated is small, statistical analysis[16] indicates that these results are applicable to sequences in general with a high level of confidence. The probability that the mean of the distribution shown in Fig. 2 is within ± n base pairs of the mean of an infinitely large sample is 0.92 for n =

1 and 0.99 for $n = 2$. Therefore, the probability of finding a 77-nucleotide RNA, random or real, without secondary structure is very small.

Transfer RNAs (average length of 77 bases) include on average 21 base pairs, but the universality of the resulting clover leaf secondary structure implicates evolutionary selection processes. If tertiary structure and base modifications are ignored, about half the natural sequences can be arranged into structures more stable than the clover leaf. (Many of these retain some elements of the clover leaf, usually the anticodon and acceptor stem. However, the existence of an acceptor stem-like feature in our random structures is an improbable event). The glycyl tRNA from *E. Coli* (Fig. 3) is the most striking example we encountered. It seems possible that selected base modification and specific tertiary interactions prevent these structures from forming under physiological conditions. However, experiments in non-physiological buffers should be interpreted with great care since at low ionic strength extended structures (for example, Fig. 3) may form which minimise the electrostatic free energy.

This structural polymorphism emphasises the need for accurate evaluation of the stability of proposed structures. Optimisation of base pairing is not the only determinant of stability since the size and number of loops is also important. Large internal loops, for example, involve considerable free energy cost and are often overlooked in stability calculations. (Examples include the acceptor stem in tRNA[17] and the flower model 'stalk' in the MS II coast protein gene[18].) When alternative structures are possible that with lowest free energy should be selected. Confirmation is sometimes possible by physical[19] or ribonuclease-digestion techniques[18].

The relationship between the presumably conflicting demands of mRNA secondary structure and amino acid sequence has been the subject of much discussion[20–22]. Our results do not support the hypothesis that amino acid sequences have evolved in part due to pressure for maintaining stable RNA base pairing[22]. Sequences generated at random exhibit pairing percentages only marginally less than those of the MSII coat protein gene. The distribution of base pairs

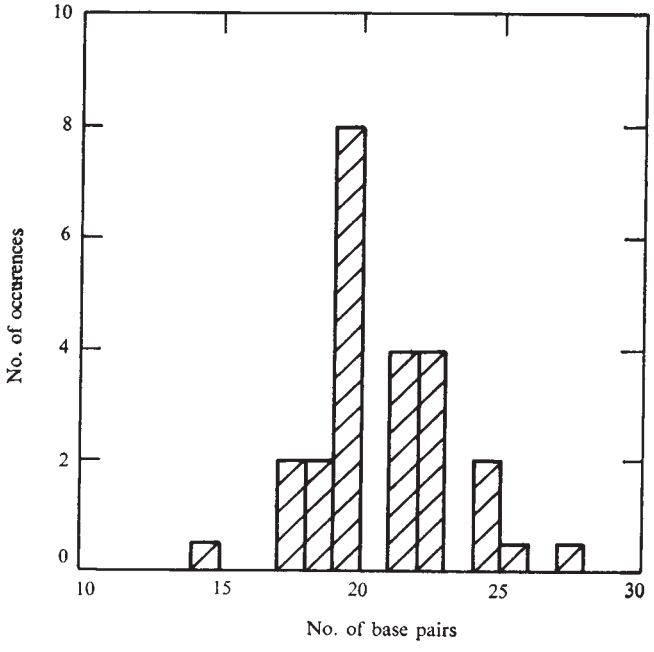


FIG. 2 A base pair distribution obtained by randomly generating twenty-five chains, each with seventy-seven base pairs. The height of the shaded area indicates the number of structures which contains the given number of base pairs. The distribution has a mean of 21.3 base pairs and a standard deviation of 2.84.

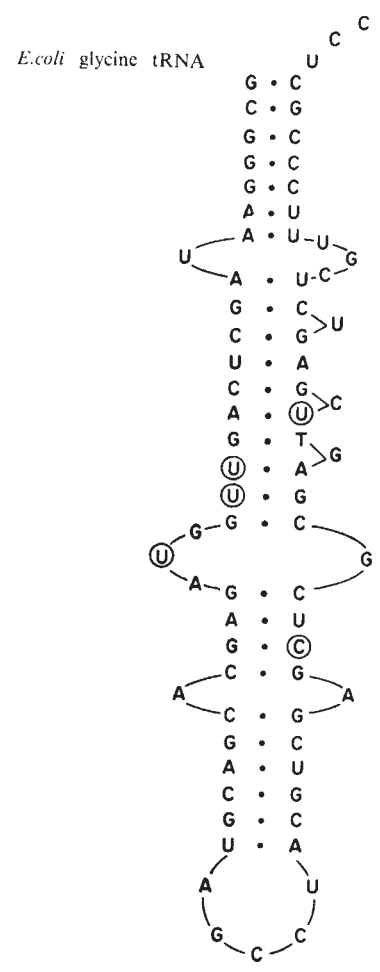


FIG. 3 A possible secondary structure from *E. coli* glycine tRNA. The circled bases are modified and do not pair in the clover leaf structure.

in this gene involving degenerate codons[22] is consistent with evolutionary pressure on the RNA sequence, or pressure on the protein amino acid sequence, or no pressure at all within statistical uncertainty. Actually, there is no obvious need for RNA to evolve toward structure of minimum free energy. Rather, specific structures should evolve for specific purposes. Defects in pairing which result in instability might even be useful in recognition functions; at the very least they would conserve energy required to disrupt base pairing during translation.

The extent of base pairing exhibited by random structures implies that all mRNAs will form secondary structures. Such high percentages arise naturally from sequences composed of four different types of bases with three allowed pairing possibilities. The absence of secondary structure in mRNA would be surprising and significant. One such example is the polyadenylate sequences present in HnRNA. There is no apparent need to postulate unusual functions or evolutionary pressure on amino acid sequence to explain the existence of stable base pairing in RNA. The results, however, do not imply that there is no evolutionary pressure on RNA sequences, but rather suggest that the existence of such pressure cannot be readily deduced from the observation that a particular messenger has 60% of its bases paired. Finally, it has been suggested that single stranded DNA may be important for control of chromosomal functions[23]. Should extensive single stranded regions of average composition exist *in vivo*, they would likely have the potential for formation of stable secondary structure.

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1 Sanger, F., *Biochem. J.*, **124**, 833 (1971).
2 Fitch, W. M., *J. molec. Evol.*, **1**, 185 (1972).
3 Adams, J. M., Jeppesen, P. G. N., Sanger, F., and Barrel, B. D., *Nature*, **223**, 1009 (1969).
4 Fiers, W., Contreras, R., DeWachter, R., Haegeman, G., Merregaert, J., Min Jou, W., and Vandenberghe, A., *Biochemie*, **53**, 495 (1971).
5 Steitz, J. A., *Nature*, **224**, 957 (1969).
6 Nichols, J. L., *Nature*, **225**, 147 (1970).
7 Lodish, H. F., and Robertson, H. D., *Cold Spring Harbor Symp. quant. Biol.*, **34**, 655 (1969).
8 Doty, P., Boedtker, H., Fresco, J. R., Haselkorn, R., and Litt, M., *Proc. natn. Acad. Sci. U.S.A.*, **45**, 482 (1959).
9 Fresco, J. R., Alberts, B. M., and Doty, P., *Nature*, **188**, 98 (1960).
10 Eigen, J., *Naturwissenschaften*, **10**, 465 (1971).
11 Tinoco, I. O., Uhlenbeck, O., and Levine, M. D., *Nature*, **230**, 362 (1971).
12 DeLisi, C., and Crothers, D. M., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2682 (1971).
13 Gralla, J., and Crothers, D. M., *J. molec. Biol.*, **73**, 497 (1973).
14 Uhlenbeck, O. C., Borer, P. N., Dengler, G., and Tinoco, jun., I., *J. molec. Biol.*, **73**, 483 (1973).
15 DeLisi, C., *Biopolymer.*, **11**, 2251 (1972).
16 Spanier, J., and Gelbart, E. M., *Monte Carlo Methods and Neutron Transport Problems*, 14 (Addison Wesley, Reading, Massachusetts, 1969).
17 DeLisi, C., *Biopolymers*, **12**, 1713 (1973).
18 Min Jou, W., Haegeman, G., Ysebgert, M., and Fiers, W., *Nature*, **237**, 82 (1972).
19 Gralla, J., Steitz, J. A., and Crothers, D. M., *Nature* (in the press).
20 Min Jou, W., Haegeman, G., and Fiers, W., *FEBS Lett.*, **13**, 105 (1971).
21 Ball, L. A., *J. thoeret. Biol.*, **36**, 313 (1972).
22 Ball, L. A., *Nature new Biol.*, **242**, 44 (1973).
23 Crick, F. H. C., *Nature*, **234**, 25 (1971).

Hepatoma—foetal Phe-tRNA also present in normal rat liver

SEVERAL groups have demonstrated independently, the presence of two isoaccepting species of Phe-tRNA in rat hepatomas and only one in normal rat liver. By use of reversed phase chromatography on RPC-2 columns[1] two groups[2–4] demonstrated an early eluting, minor peak of Phe-tRNA in Morris hepatomas 5123C and 5123D, which was not seen in normal rat liver tRNA, while one group[5,6] reported the same extra peak in hepatoma 9618A but not in 3924A. We too have observed this minor peak of Phe-tRNA in Morris hepatomas 5123D, 7777 and 7800 using RPC-2 chromatography. Figure 1*a* shows a typical RPC-2 pattern for these three hepatomas all of which had a minor Phe-tRNA peak containing 14–15% of the total acid-precipitable counts. Recently Gonano *et al.* reported a similar peak of Phe-tRNA in foetal rat liver which cochromatographs on RPC-2 with the minor peak from hepatoma 5123C[7].

Recent work in this laboratory, comparing aminoacyl-tRNAs from mouse and rat tumours, has used another reversed phase column system, RPC-5[8], and fresh isolations of tRNA and aminoacylating enzymes from tumours and normal tissues. The RPC-5 profiles were basically in agreement with those from RPC-2 runs of older preparations and

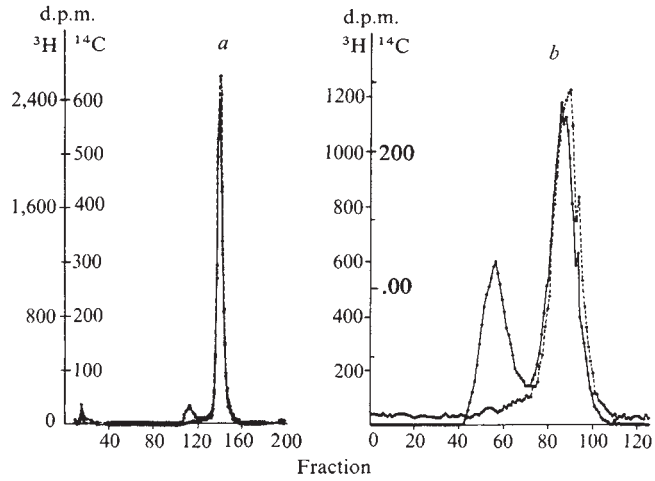


FIG. 1 *a*, Elution pattern from an RPC-2 column[4] on which ^{3}H-Phe-tRNA from Morris hepatoma 7800 (– – ○ – –) was cochromatographed with ^{14}C-Phe-tRNA from normal adult Buffalo rat liver (—●—). *b*, Elution pattern from an RPC-5 column[8] on which ^{3}H-Phe-tRNA from normal adult Buffalo rat liver (– – ○ – –) was cochromatographed with ^{14}C-Phe-tRNA from Morris hepatoma 7777 (—●—). Phe-tRNAs were prepared by incubating Sephadex G-100-purified[13] 4S RNA with aminoacylating enzymes (see below) in a reaction mixture containing 5mM Na_2ATP (neutralised), 100 mM Tris-HCl, *p*H 7.6, 10 mM $MgCl_2$, 14 mM β-mercaptoethanol, 0.25 mM each of nineteen amino acids (minus phenylalanine) and 4×10^{-5}–7×10^{-5} M ^{3}H-L-phenylalanine or ^{14}C-L-phenylalanine for 30 min at 37° C. The aminoacylating enzymes were prepared from an homogenate of liver or tumour in medium A1 (0.25 M sucrose, 0.006 M $MgCl_2$, 0.015 M Tris-HCl, *p*H 7.6, 0.8M KCl and 0.014 M β-mercaptoethanol) from which the cellular membranes and ribosomes had been removed by centrifugation at 100,000*g* for 2 h. This was absorbed to and eluted from a column of DEAE cellulose by the method of Muench and Berg[9] and stored in 50% glycerol at —20° C for months (DEAE enzyme), or the S-100 was merely passed over a small column of Sephadex G-10 equilibrated with medium A1 minus sucrose and stored in 50% glycerol at —20° C (G-10 enzyme). After three phenol extracts the labelled Phe-tRNAs were precipitated with ethanol at —20° C, dissolved in column buffer, desalted on Sephadex G-25, mixed and applied to the column specified. The RPC-2 column (1 cm × 240 cm) was eluted at 15° C with a 2 l linear gradient from 0.35 M to 0.65 M NaCl in 0.01 M Na acetate and 0.01 M Mg acetate, *p*H 4.5. Fractions (10 ml) were collected and precipitated with 1ml 50% trichloroacetic acid at 4° C, and the precipitates were collected on Millipore filters which were dried, treated with 0.2 ml 1 M KOH for 5 min at room temperature, neutralised with 1 M HCl and counted in a liquid scintillation counter. Three replicate counts were averaged and converted to absolute activity on an IBM 1620 computer. The RPC-5 column (0.5 cm × 50 cm) was eluted at 22° C with a 400 ml linear gradient from 0.5 M to 0.65 M NaCl in 0.01 M Na acetate, 0.01 M Mg acetate and 0.001 M β-mercaptoethanol. Fractions (2.5 ml) were collected, precipitated with 2.5 ml 10% trichloroacetic acid at 4° C, and processed as above.

showed an early eluting peak of Phe-tRNA in hepatomas but not in normal liver (Fig. 1*b*). While conditions for optimal aminoacylation of phenylalanine to each batch of tRNA were being retested, the DEAE cellulose preparation of aminoacylating enzymes (DEAE enzymes)[9] from liver or tumour was found not to be as effective in the extent of aminoacylation as was a cruder preparation made by passing a 100,000*g* supernatant of tissue homogenate over a small column of Sephadex G-10 (G-10 enzymes). The optimal aminoacylating conditions were the same for enzymes prepared by either procedure. RPC-5 chromatography of tRNA from all three hepatomas aminoacylated with G-10 enzymes showed a more prominent early eluting peak of Phe-tRNA (Fig. 1*b*) than that found by RPC-2 chromatography of tRNA aminoacylated with DEAE enzyme (Fig. 1*a*). Further, liver tRNA aminoacylated using the G-10 enzyme showed

the presence of a prominent minor peak of Phe-tRNA which had not been seen when the DEAE enzyme preparation was used (Fig. 2).

Because a loss of activity might have occurred during storage of the DEAE enzyme preparation at −20° C in 50% glycerol, a fresh preparation was made from normal rat liver by the procedure used before. Indeed the fresh enzyme was more active in aminoacylating phenylalanine to liver tRNA but not to the extent of a fresh or stored G-10 enzyme preparation. The fresh DEAE enzyme preparation also revealed a very small minor Phe-tRNA peak (Fig. 2), but this was always smaller than the minor peak revealed when the same liver tRNA was aminoacylated with G-10 enzyme.

The G-10 enzyme preparation contained some endogenous tRNA, but the earlier eluting peak of Phe-tRNA was not found in it. Mixtures of DEAE and G-10 enzyme preparations yielded Phe-tRNA identical in amount and pattern to that produced by G-10 enzyme only, ruling out the presence of an inhibitor in the DEAE enzyme.

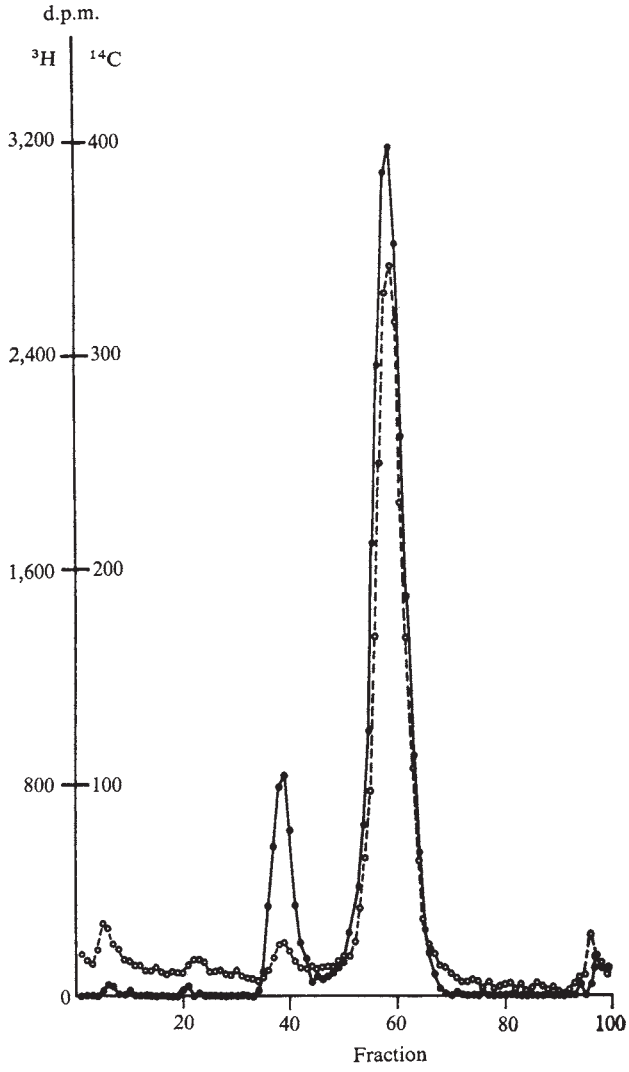


FIG. 2 Elution pattern from an RPC-5 column showing the effect of aminoacylating enzymes prepared by two methods on the pattern of Phe-tRNA prepared from normal rat liver. tRNA was aminoacylated with ³H-L-phenylalanine using liver enzymes freshly eluted from DEAE cellulose (stored at 20° C for less than 7 d) (– – ○ – –) and mixed with tRNA from the same source which had been aminoacylated with ¹⁴C-L-phenylalanine using liver cytosol merely passed over Sephadex G-10 (—●—) (see Fig. 1 legend for conditions of enzyme preparations and the aminoacylation reaction). The RPC-5 column (0.5 cm × 30 cm) was developed under high pressure[14] at 22° C with a 150 ml gradient of NaCl from 0.5 M to 0.8 M in 0.01 M Na acetate, 0.01 M Mg acetate and 0.001 β-mercaptoethanol. Fractions (1.2 ml) were collected directly in glass vials and counted in a liquid scintillation counter. Two replicate counts were averaged, and absolute activity was calculated in a PDP-11 computer.

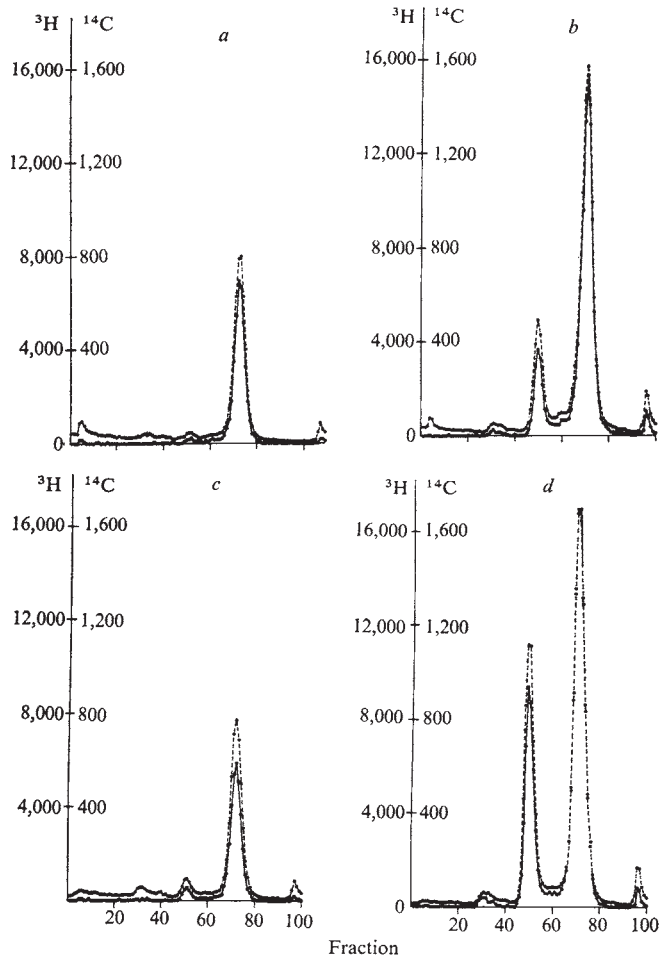


FIG. 3 Four elution patterns from RPC-5 columns using conditions indicated in the legend for Fig. 2. tRNA (30 μg) from normal rat liver (*a* and *b*) or Morris hepatoma 5123D (*c* and *d*) were aminoacylated with ³H-L-phenylalanine and chromatographed with 45 μg tRNA from the source that had been aminoacylated with ¹⁴C-L-phenylalanine. Reaction conditions are given in the legend for Fig. 1, and the type and source of enzyme used was as follows. *a*: – –○– –, ³H-Phe, liver RNA, liver DEAE enzyme; —●—, ¹⁴C-Phe, liver RNA, 5123 DEAE enzyme. *b*; —○—, ³H-Phe, liver RNA, 5123 G-10 enzyme;—●—, ¹⁴C-Phe, liver RNA, liver G-10 enzyme. *c*: —○— ³H-Phe, 5123 RNA, liver DEAE enzyme; – ● –, ¹⁴C-Phe, 5123 RNA, 5123 DEAE enzyme. *d*: – –○– –, ³H-Phe, 5123 RNA, 5123 G-10 enzyme; —●—, ¹⁴C-Phe, 5123 RNA, liver G-10 enzyme.

The effect of the two enzyme preparations on tumour tRNA was then studied. Morris hepatoma 5123D and normal rat liver were used as the sources of tRNA and enzymes. Identical amounts of liver tRNA (Fig. 3*a* and *b*) and tumour tRNA (Fig. 3, *c* and *d*) were aminoacylated with DEAE and G-10 enzymes from liver and tumour in all four combinations and plotted on the same scale. The same type of enzyme preparation, whether from normal or neoplastic tissue, gave the same extent and pattern of aminoacylation of phenylalanine to a particular tRNA preparation. G-10 enzymes increased the total incorporation into the major peak of Phe-tRNA two to three-fold over DEAE enzymes while increasing the incorporation into the minor peak more than fifteen-fold, such that a minor peak not seen in liver tRNA charged with aged DEAE enzyme became very prominent after G-10 enzyme had been used. Furthermore, there

was a distinct difference in the prominence of the minor Phe-tRNA from normal liver and from hepatomas no matter which aminoacylating enzyme was used, but it no longer seems to be accurate to refer to a tumour-specific peak of Phe-tRNA or one completely absent in normal rat liver. In fact, the presence of the minor peak of Phe-tRNA in normal liver has been reported by Tidwell *et al.*[10] in a detailed study of normal and regenerating rat liver.

Possibly a more interesting aspect of these findings is the difference between the two enzyme preparations that enables the minor peak of normal Phe-tRNA to be demonstrated. Work is underway to investigate several possibilities, for example, that DEAE cellulose purification removes or inactivates one of several Phe-tRNA sythetases or a tRNA-modifying enzyme. Tumour tissue may contain such an excess of this factor that the DEAE cellulose step does not remove all its activity.

These investigations have not yet included a study of foetal tRNA, but we suggest that the striking abundance of the early-eluting Phe-tRNA peak in foetal rat liver, reported by Gonano *et al.*[7], may also be associated with a great abundance of that activity we find deficient in our DEAE enzyme but abundant in the G-10 preparations. The association of neoplasia and foetal gene activation is particularly provocative and this increased amount of a normally minor Phe-tRNA in foetal and neoplastic hepatic tissue should be explored further, remembering that equally significant changes may be occurring simultaneously in the enzymes involved in tRNA aminoacylation. Another product of foetal liver, α-foetoprotein, has also been associated with hepatomas in several species. Apparently α-foetoprotein is secreted by only a limited number of rat hepatomas[11], with abnormally high serum levels reported for Morris hepatomas 3924A and 7777 but not for 9618A, 5123D or 7800, all of which (except 3924A[5]) have a prominent peak of the minor Phe-tRNA. Experiments with regenerating rat liver also suggest a transient increase in α-foetoprotein secretion[12], but only normal amounts of the minor Phe-tRNA peak have been reported 12, 18 and 24 h after partial hepatectomy[10]. We have not found increases in the minor Phe-tRNA in regenerating liver taken 1, 2, 3, 7, and 10 d after partial hepatectomy. So although both the minor Phe-tRNA and α-foetoprotein may represent increased activation of genes primarily associated with embryogenesis or rapid growth conditions, a direct correlation does not exist between them.

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1 Weiss, J. F., and Kelmers, A. D., *Biochemistry*, **6**, 2507 (1967).
2 Gonano, F., and Chiarugi, V. P., *Exp. molec. Pathol.*, **10**, 99 (1969).
3 Volkers, S. A. S., and Taylor, M. W., *Biochemistry*, **10**, 488 (1971).
4 Gonano, F., Chiarugi, V. P., Pirro, G., and Marini, M., *Biochemistry*, **10**, 900 (1971).
5 Volkers, S. A. S., and Taylor, M. W., *Biochem. biophys. Acta.*, **254**, 415 (1971).
6 Taylor, M. W., Volkers, S. A. S., Choe, B. K., and Zeikus, *Cancer Res.*, **31**, 688 (1971).
7 Gonano, F., Pirro, G., and Silvetti, S., *Nature new Biol.*, **242**, 236 (1973).
8 Pearson, R. L., Weiss, J. F., and Kelmers, A. D., *Biochem. biophys. Acta.*, **228**, 770 (1971).
9 Muench, K. H., and Berg, P., *Proc. Nucleic Acid Res.*, **1**, 375 (1966).
10 Tidwell, T., Bruce, B. J., and Griffin, A. C., *Cancer Res.*, **32**, 1002 (1972).
11 Sell, S., Jalowayski, I., Bellone, C., and Wepsic, H. T., *Cancer Res.*, **32**, 1184 (1972).
12 Abelev, G. I., *Adv. Cancer Res.*, **14**, 295 (1971).
13 Mushinski, J. F., *Biochemistry*, **10**, 3917 (1971).
14 Kelmers, A. D., and Heatherly, D. E., *Anal. Biochem.*, **44**, 486 (1971).

Activity of DNA templates during cell division and cell differentiation

The rates of cellular RNA and DNA synthesis decline during the mitotic stages of cell division[1] and during cell differentiation[2]. The molecular mechanisms mediating such a decline may include changes in the size of mononucleotide pools, changes in RNA and DNA polymerase activity, or changes in DNA template activity[3], but previous studies have not distinguished among these possibilities. We have developed a high-resolution electron microscopic technique for directly analysing DNA template activity within living cells[4], and now report its application to individual cells undergoing division and differentiation in human bone marrow.

Bone marrow spicules[5] and peripheral blood cells[6] were isolated under sterile conditions from patients as previously described, and were subjected to the analysis of active DNA templates while in the living state[4]. Acridine orange was used as previously described[4] to probe for the location and number of active DNA templates within each cell[5]. The location of each probe site was determined by electron microscopic visualisation of the electron-dense reaction product formed between acridine orange and osmic acid[5]. Control experiments omitting either the acridine orange or the DNase steps from the reaction sequence did not reveal any reaction product[5]. Positive cells revealed large (>0.1 μm) or small (0.025–0.1 μm) reaction products localised exclusively within the nuclear euchromatin complexes[5]. These probe sites were counted within each cell by one observer, and another observer independently classified the stage of cell division or of cell differentiation, using the ultrastructural criteria previously reported for dividing cells[7], differentiating granulocytes[8], and differentiating erythrocytes[9].

A total of fifty dividing normal bone marrow cells were analysed (Table 1). These analyses revealed a progressive decrease in the number of probe sites per cell through the early and late stages of prophase, to an absence of any probe sites in metaphase, anaphase and early telophase, with a subsequent rapid increase in the number of probe sites per cell in late telophase, returning to a basal level in interphase.

Similarly a total of 123 normal differentiating granulocyte precursor cells and 176 normal differentiating erythrocyte precursor cells (Table 2) were analysed. These analyses revealed a progressive decrease during the course of normal cell differentiation in the percentage of cells containing either large or small probe sites. In addition, these analyses revealed a progressive decrease during cell differentiation in the number of large probe sites per positive cell, as well as a progressive decrease in the fraction of all probe sites represented

Table 1 Probe counts in dividing bone marrow cells

Stage of cell division	Mean probe count per cell
Early prophase	35.3
Late prophase	14.8
Metaphase, anaphase, early telophase	0
Late telophase	85.0
Interphase	35.1

TABLE 2 Probe counts within differentiating marrow cells

Granulocytes Stage of cell differentiation	% Cells containing Large probes	% Cells containing Small probes	Mean probe count per positive cell Large probes	Mean probe count per positive cell (Large probes)/(total probes)
Promyelocytes	91.5	100.	9.55	0.19
Myelocytes	85.9	96.4	8.75	0.18
Metamyelocytes	47.6	85.7	3.9	0.05
Band granulocytes	0	20.0	0	0.0
Segmented granulocytes	0	12.2	0	0.0
Erythrocytes				
Proerythroblasts	100	100	16.0	0.65
Early erythroblasts	84.7	91.6	8.5	0.32
Late erythroblasts	14.8	17.2	3.4	0.20
Nucleated erythrocytes	0	8.3	0	0

by large probe sites (Table 2). These observed decreases in DNA template activity during the course of normal bone marrow cell differentiation are statistically highly significant ($P < 0.01$) by Mann's test for trend significance[10].

Independent analyses have previously revealed a direct correlation between DNA template activity as measured by probe sites within isolated chromatin, and the rate of RNA synthesis by such isolated chromatin when supplied with excess amounts of exogenous RNA polymerase and monoribonucleotides[11]. The studies reported here measuring probe sites within intact living cells similarly correlate closely with previous independent studies demonstrating a progressive decrease in RNA and DNA synthesis and cell division during normal bone marrow cell differentiation[2]. These quantitative data suggest that a progressive restriction of DNA templates is an important mechanism during normal cell division and bone marrow cell differentiation[12]. We are currently studying the molecular species[13] mediating such restriction and release of DNA template activity during normal[6] and leukaemic cell division and differentiation[14].

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1 Simmons, T., Heywood, P., and Hodge, L., *J. cell Biol.*, **59**, 150 (1973).
2 Feinendegan, L. E., Bond, V. P., Cronkite, E. P., and Hughes, W. L., *Ann. N.Y. Acad. Sci.*, **113**, 727 (1964).
3 Keshgegian, A. A., Meisner, L. F., and Frenster, J. H., in *Proc. fourth Leukocyte Culture Conf.*, (edit. by McIntyre, O. R.), 361 (Appleton-Century-Crofts, New York, 1971).
4 Frenster, J. H., *Nature new Biol.*, **236**, 175 (1972).
5 Frenster, J. H., *Cancer Res.*, **31**, 1128 (1971).
6 Frenster, J. H., Best, W. R., and Winzler, R. J., *Proc. Soc. exp. Biol. Med.*, **98**, 887 (1958).
7 Robbins, E., and Gonatas, N. K., *J. cell Biol.*, **21**, 429 (1964).
8 Anderson, D. R., *J. Ultrastruc. Res. Suppl.*, **9**, 5 (1966).
9 Bessis, M. C., and Breton-Gorius, J., *Blood*, **19**, 635 (1962).
10 Hollander, M., and Wolfe, D. A., *Non-Parametric Statistical Methods* (Wiley, New York, 1973).
11 Seligy, V. L., and Lurquin, P. F., *Nature new Biol.*, **243**, 20 (1973).
12 Frenster, J. H., and Herstein, P. R., *New Engl. J. Med.*, **228**, 1224 (1973).
13 Frenster, J. H., *Nature*, **208**, 1093 (1965).
14 Frenster, J. H., *The Cell Nucleus* (edit. by Busch, H.) 1, 565–580 (Academic Press, New York, 1974).

Functional instability of T7 early mRNA

IT has been known for some time that mRNA of bacteriophage T7 is stable. Summers[1] measured the decay of acid-insoluble radioactivity in the mRNA after new synthesis was stopped with actinomycin in T7-infected, actinomycin-permeable *Escherichia coli* cells. The halflife of T7 mRNA was estimated greater than 25-30 min at 30° C compared with about 2.5 min for *E. coli* mRNA. Marrs and Yanofsky[2] showed that the mRNA transcribed from the tryptophan operon is degraded normally in T7-infected *E. coli* but T7 mRNA is stable, measured by hybridisation with T7 DNA. In these experiments only chemical stability of T7 mRNA was studied in terms of acid-insoluble amount or DNA-hybridisable amount and no information was obtained on the functional integrity of the mRNA.

During the late period of T7 infection only late proteins are synthesised. If T7 early mRNAs are functionally stable and present in the cell together with late mRNA, preferential synthesis of late proteins must depend on some sort of translational control exerted at the level of protein synthesis. However, no discriminatory translational control against early mRNA in T7-infected cells has been detected (submitted for publication). Therefore, we have examined the functional and structural integrity of T7 early mRNA produced by an amber mutant, T7 *am*193. Since the amber mutation is in gene 1 coding for T7-specific RNA polymerase[3], this mutant phage infection on a non-permissive host makes only early mRNA but no late mRNA.

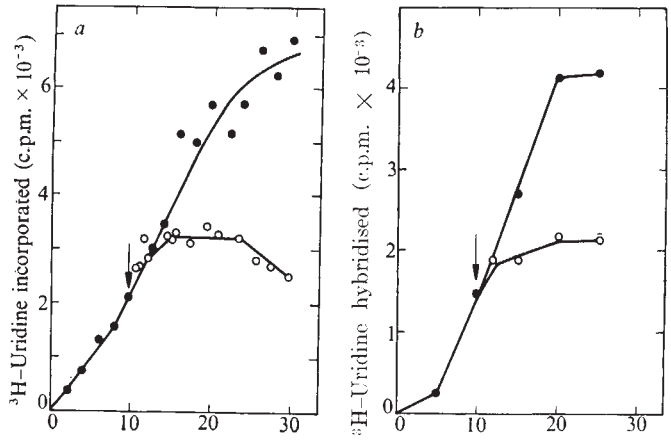


FIG. 1 RNA synthesis in ultraviolet-irradiated D10 F$^-$ cells infected with T7 *am*193. *a*, ^{3}H-uridine incorporation into RNA; *b*, T7 DNA-hybridisable ^{3}H-uridine radioactivity in RNA. *a*, *E. coli* D10 F$^-$ was grown in M9 medium at 30° C to a cell density of 6×10^8 ml^{-1}. The culture was chilled and transferred to a glass Petri dish, irradiated for 7 min with two Sylvania germicidal lamps G15TB at 25 cm, then shaken at 30° C for 5 min. Survival was less than 10^5 ml^{-1}. The irradiated culture was then infected with T7 *am*193 at a multiplicity of 10 and labelled with ^{3}H-uridine (5 μCi in 3 μg ml^{-1}). After 10 min at 30° C, the culture was divided into two. One portion was diluted with an equal volume of pre-warmed medium, the other with pre-warmed medium containing 800 μg ml^{-1} rifampicin. Samples of 0.025 ml before dilution and 0.05 ml after dilution were withdrawn and acid-insoluble radioactivity was counted. ●, ^{3}H-Uridine incorporation in T7 *am*193-infected cells; ○, ^{3}H-uridine incorporation in T7 *am*193-infected cells treated with rifampicin at 10 min (arrow). *b*, Experimental conditions were the same as described in (*a*) except that 20 μCi in 1 μg ml^{-1} of ^{3}H-uridine was added. Samples of 5 ml before and 10 ml after dilution were withdrawn and radioactive RNA was prepared from each. 1.6 μg of RNA was hybridised to T7 DNA bound to filters[12]. A mean value of duplicate samples was plotted. ●, Hybridisable ^{3}H-count in RNA from T7 *am*193-infected cells; ○, hybridisable ^{3}H-count in RNA from T7 *am*193-infected cells treated with rifampicin at 10 min (arrow).

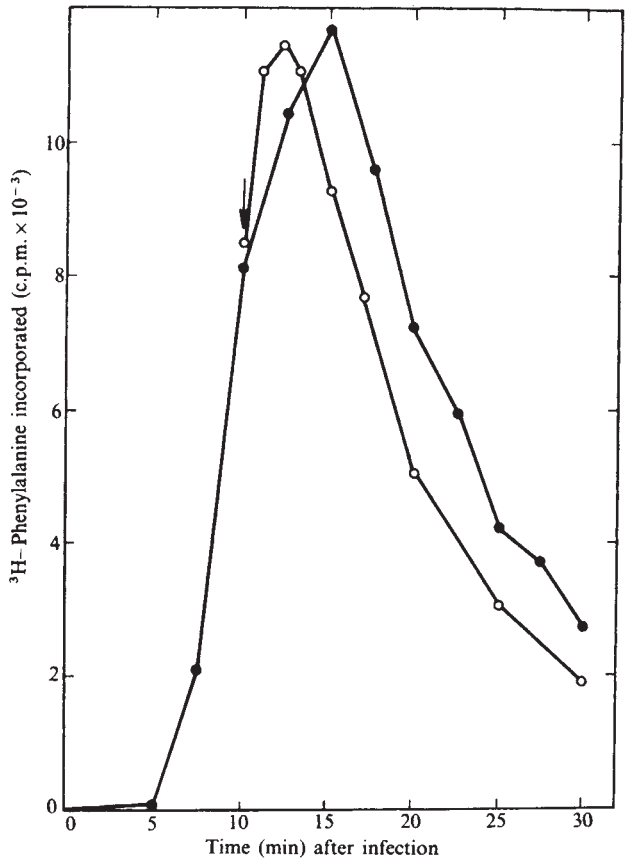


FIG. 2 Functional instability of T7 *am*193 mRNA in directing *in vitro* protein synthesis. Ultraviolet-irradiated *E. coli* D10 F$^-$ was infected with T7 *am*193 as described in the Fig. 1 legend. RNA was extracted from the samples withdrawn at indicated times and the messenger activity of the RNA to direct *in vitro* protein synthesis was tested. The cell-free protein-synthesising system contained 5 ^{260}A units of IS_{30} (pre-incubated cell-free extract) from uninfected D10 cells and 5 μCi ^{3}H-phenylalanine in a total volume of 0.25 ml. RNA (40 μg) from each sample was added as messenger. Incubation was at 35° C for 30 min and hot acid-precipitable radioactivity was counted. ●, Messenger activity of RNA from T7 *am*193-infected cells; ○, messenger activity of RNA from T7 *am*193-infected cells treated with rifampicin at 10 min (arrow).

Our results described here confirmed the chemical stability of T7 early mRNA. We found, however, that functional activity of T7 early mRNA, tested in an *in vitro* protein-synthesising system, decayed at a rather rapid rate, a half-life about 6.5 min at 30° C. We believe that the functional decay is related to a loss of structural integrity of T7 early mRNA without losing much chemical quantity, because acrylamide-agarose gel electrophoresis revealed that the size of T7 early mRNA became smaller parellel to the loss of functional activity.

We used ultraviolet-irradiated bacteria in order to reduce host RNA synthesis after T7 infection. *E.* coli D10 F$^-$, derived from D10 by acridine orange treatment (submitted for publication), was irradiated and then infected with T7 *am*193. In ultraviolet-irradiated cells, T7-directed synthesis of macromolecules proceeded somewhat slower than in normal cells. ^{3}H-Uridine incorporation into RNA is shown in Fig. 1*a*. Ultraviolet-irradiated cells showed an accumulation of T7 early mRNA. Addition of rifampicin effectively shut off new initiation of RNA synthesis but the acid-insoluble radioactivity of the RNA declined only slightly after the addition of rifampicin.

Figure 1*b* shows the accumulation of T7 early mRNA which can be hybridised to T7 DNA. Ultraviolet-irradiated D10 F$^-$ cells were infected with T7 *am*193 and labelled with ^{3}H-uridine. RNA was extracted from the cells taken at indicated times. A small amount of RNA synthesised by ultraviolet-irradiated cells without T7 infection did not have any hybridisable radioactivity (not shown in Fig. 1*b*). Confirming the result of Marrs and Yanofsky[2], we observed the stability of hybridisable T7 early mRNA even in the presence of rifampicin.

When T7 early mRNA was isolated from ultraviolet-irradiated, T7 *am*193-infected cells and tested for functional activity in an *in vitro* protein-synthesising system, we found that T7 early mRNA lost functional integrity at a rather rapid rate (Figure 2). RNA was extracted from aliquots of the infected culture at various times and added to a protein-synthesising system prepared from uninfected D10 F$^-$ cells, and ^{3}H-phenylalanine incorporation was measured. Protein synthesis-directing activity of the RNA decayed at a rapid rate after 15 min of infection even in the absence of rifampicin (Fig. 2). With rifampicin, functional decay of the RNA started earlier. The rate of decay with or without rifampicin was, however, similar.

Figure 3 shows f-met-tRNA binding to ribosomes directed by the RNA taken from ultraviolet-irradiated, T7 *am*193-infected cells. Salt-washed ribosomes and crude initiation factors were prepared from uninfected D10 F$^-$ cells. Functional activity of T7 early mRNA in directing f-met-tRNA binding also decayed rapidly (compare with Fig. 3). Decay again started earlier in the presence than in the absence of rifampicin.

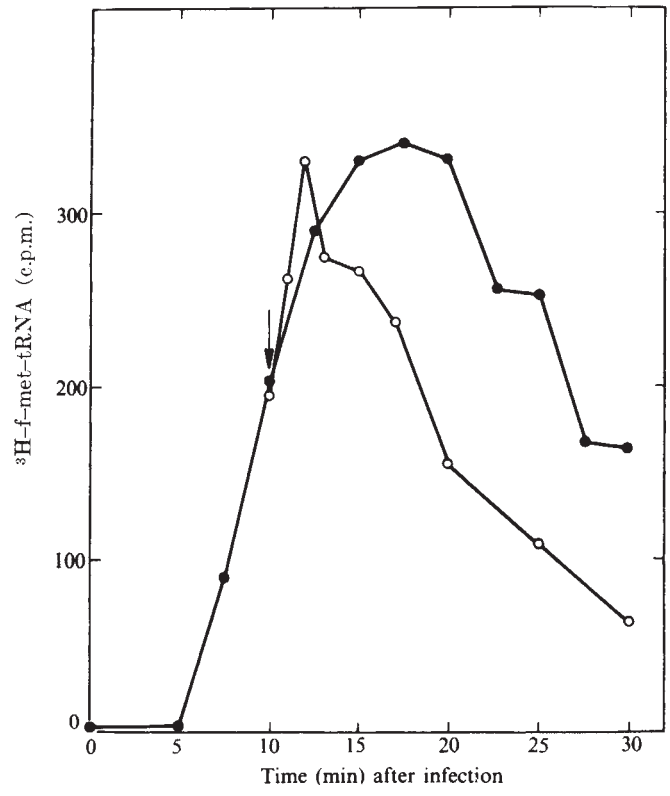


FIG. 3 Functional instability of T7 *am*193 mRNA in directing the binding of f-met-tRNA to ribosomes. The same RNA samples from the experiment of Fig. 2 were used to direct initiation factor-dependent f-met-tRNA binding to ribosomes. The reaction mixture (submitted for publication) contained 75 μg of 1 M NH_4Cl-washed ribosomes and 8 μg unfractionated initiation factors from uninfected D10 F$^-$ cells and ^{3}H-f-met-tRNA (1,500 c.p.m. pmol^{-1}) in a total volume of 0.2 ml. RNA (54 μg) from each sample was added as messenger. Incubation was at 35° C for 15 min and radioactivity of ^{3}H-f-met-tRNA retainable on a Millipore filter was counted. ●, Messenger activity of RNA from T7 *am*193-infected cells; ○, messenger activity of RNA from T7 *am*193-infected cells treated with rifampicin at 10 min (arrow).

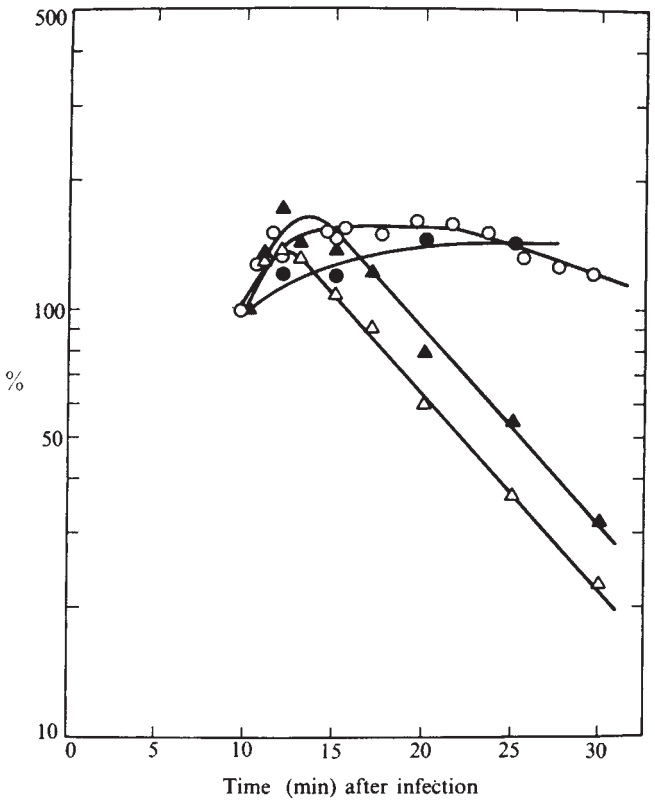


FIG. 4 Chemical stability and functional decay of T7 mRNA. Results of Figs 1, 2 and 3, obtained from the ultraviolet-irradiated cells infected with T7 *am*193 and treated with rifampicin at 10 min, were replotted. The 100% value represents the value at the time of rifampicin addition (10 min after infection). ●, Acid-insoluble radioactivity in RNA (Fig. 1*a*); ○, T7 DNA-hybridisable radioactivity in RNA (Fig. 1*b*); △, messenger activity in RNA to direct protein synthesis (Fig. 2); ▲, messenger activity in RNA to direct f-met-tRNA binding (Fig. 3).

Figure 4 summarises the above results obtained in the presence of rifampicin. After the addition of rifampicin at 10 min of infection, when there was extensive synthesis of phage RNA, there was a slight increase of functional activity due to the residual synthesis of RNA expected from the mode of action of rifampicin. Thereafter, a rapid decay of functional activity in directing total protein synthesis and also initiation factor-dependent f-met-tRNA binding took place. Both decay curves are almost parallel and give an estimate of the functional halflife of T7 early mRNA as about 6.5 min at 30° C. On the other hand, the acid-insoluble and hybridisable amounts of the RNA remained stable.

A loss of structural integrity in T7 early mRNA was found by electrophoresis of the RNA in polyacrylamide gel containing agarose. This method gave a much better resolution for the T7 early mRNA species[4] than the procedure previously used. RNA extracted from ultraviolet-irradiated D10 F⁻ cells infected with T7 *am*193 was labelled with ^{3}H-uridine. Portions of these RNA samples were used for hybridisation experiments (Fig. 1*b*). Electrophoretic patterns in Figs 5 and 6 show that T7 early mRNAs were partially degraded as time progressed in the absence (Fig. 5) and presence (Fig. 6) of rifampicin. It seems that more smaller size RNAs were produced, at the expense of full size RNAs. Since total radioactivity in the RNA samples taken at different times in the presence of rifampicin was almost the same (see Fig. 1*a* and *b*) and relatively large RNAs remained after a long time, it is unlikely that an extensive nucleolytic degradation had taken place. Figure 5 and 6 show that the largest species of T7 early mRNA, gene 1 messenger, overlapping with the marker 23S rRNA (see ref. 4) at 10 min (Figs 5*a* and 6*a*) shifts to a slightly smaller size as time proceeds (Figs 5 and 6–*d*).

Our results show that structural integrity and functional activity of T7 early mRNA are closely related. Loss of structural integrity, not detected by measuring chemical amount or hybridisability, plays an important role in messenger function. This warns that hybridisable radioactivity in mRNA is not necessarily a measure of functional activity of the mRNA. Our results also explain why only late proteins are produced in the presence of chemically stable early mRNA in late T7 infection.

Whereas in *E. coli*, mRNAs appear to have identical or similar decay halflives in functional activity and chemical amount (see ref. 5), a similar difference between hybridisability and functional stability in mRNA was found in single-stranded DNA phage S13 (ref. 6). Messenger RNAs of other phages, ΦX174 (ref. 7) and M13 (ref. 8), were shown to have relatively long halflives in terms of hybridisability. It would be interesting to know whether these phage mRNAs show a difference between functional and chemical stability.

At the moment we have no direct information as to why T7 early mRNA is chemically stable. Since host mRNA (at least tryptophan mRNA) decays normally in T7-infected cells[2], T7-directed modification of general mRNA degradation system of the host is unlikely. It is conceivable that T7 mRNA may have structural features, such as extensive base-pairings

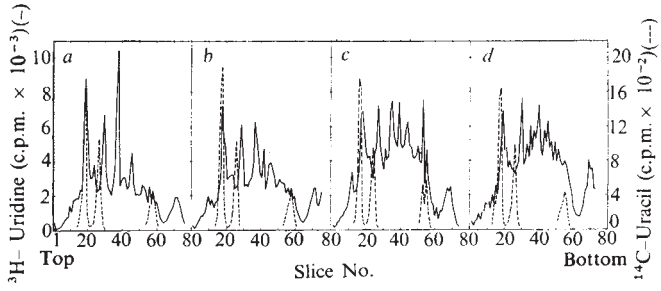


FIG. 5 Polyacrylamide-agarose gel electrophoretic pattern of T7 *am*193 mRNA. The ^{3}H-labelled RNA samples, used in the hybridisation experiment (Fig. 1*b*), were analysed by gel electrophoresis. RNA samples (about 1.7 × 10^5 c.p.m. per sample) were mixed with ^{14}C-labelled total *E. coli* RNA and subjected to electrophoresis in 2.5% acrylamide-0.5% agarose gel for 1.5 hours at 5 mA per gel[4,13]. Gels were frozen and sliced into 1 mm pieces. Radioactivity of ^{3}H in each slice was counted[4]. The direction of electrophoresis was from left to right. *a*, T7 *am*193 RNA from ultraviolet-irradiated cells 10 min after infection; *b*, 15 min; *c*, 20 min; *d*, 25 min; —, ^{3}H-labelled T7 *am*193 RNA; ---, ^{14}C-labelled marker *E. coli* RNA, 23S, 16S, and 4S, from left to right.

or special base sequences, such that host mRNA degradation enzyme(s) fail to attack the phage mRNA. An endonucleolytic enzyme, identical or similar to RNase III[9], is required to process a large single piece of RNA synthesised *in vitro* by *E. coli* RNA polymerase on T7 DNA template. Dunn and Studier[10] showed that this large size RNA, a transcript of entire T7 early genes, can be cleaved to yield several pieces of smaller size RNAs corresponding to the size of *in vivo* T7 early mRNAs. Since RNase III is a double stranded RNA-specific endonuclease, this finding indicates some degree of base pairing in T7 early mRNA.

A preliminary experiment using wild-type T7 phage, instead of T7 *am*193, indicated that the functional activity of both early and late mRNA decays rapidly. Recently, Hagen and Young[11] reported that T7 lysozyme mRNA (transcript of one of the lage genes) is unstable in directing *in vitro* synthesis of lysozyme.

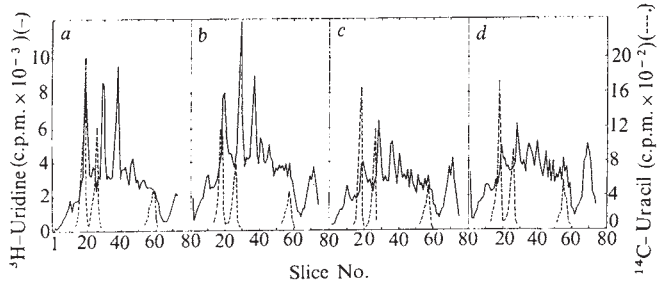


FIG. 6 Polyacrylamide-agarose gel electrophoretic pattern of T7 *am*193 mRNA after rifampicin treatment. RNA samples were as used in the hybridisation experiment shown in Fig. 1*b*. Electrophoresis was carried out as described in the legend for Fig. 5. *a*, T7 *am*193 RNA from ultraviolet-irradiated cells 12 min after infection (rifampicin was added at 10 min); *b*, 15 min; *c*, 20 min; *d*, 25 min; —, ^{3}H-labelled T7 *am*193 RNA; – – –, ^{14}C-labelled marker *E. coli* 23S, 16S, and 4S, from left to right.

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1 Summers, W. C., *J. molec. Biol.*, **51**, 671 (1970).
2 Marrs, B. L., and Yanofsky, C., *Nature new Biol.*, **234**, 168 (1971).
3 Studier, F. W., *Virology*, **39**, 562 (1969).
4 Minkley, E. G., and Pribnow, D., *J. molec. Biol.*, **77**, 255 (1973).
5 Adesnik, M., and Levinthal, C., *Cold Spring Harbor Symp. quant. Biol.*, **35**, 451 (1970).
6 Puga, A., Barrás, M.-T., Tessman, E. S., and Tessman, I., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2171 (1973).
7 Hayashi, Y., and Hayashi, M., *Cold Spring Harbor Symp. quant. Biol.*, **35**, 171 (1970).
8 Jaenish, R., Jacob, E., and Hofschneider, P. H., *Nature*, **227**, 59 (1970).
9 Robertson, H. D., Webster, R. E., and Zinder, N. D., *J. biol. Chem.*, **243**, 82 (1968).
10 Dunn, J. J., and Studier, F. W., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1559 (1973).
11 Hagen, F., and Young, E. T., *Virology*, **55**, 231 (1973).
12 Gillespie, D., and Spiegelman, S., *J. molec. Biol.*, **12**, 829 (1965).
13 Peacock, A. C., and Dingman, C. W., *Biochemistry*, **7**, 668 (1968).

Hydrophobic bonding and accessible surface area in proteins

THE hydrophobic bond is the term used by Kauzmann[1] to describe the gain in free energy on the transfer of non-polar residues from an aqueous environment to the interior of proteins. This has been accepted as one of the major forces involved in the folding of proteins. The exact origin of the energy of the hydrophobic bond is controversial[2], but empirical values have been derived for 10 protein residue side chains by Nozaki and Tanford[3] who measured the solubility of amino acids in the organic solvents ethanol and dioxane.

For an atomic group in a protein to interact with the solvent it must be able to form Van der Waals contracts with water molecules. The concept of accessible surface area has been introduced by Lee and Richards[4] to describe quantitatively the relationship of proteins to solvent. The accessible surface area of an atomic group is defined as the 'area on the surface of a sphere radius R, on each point of which the centre of a solvent molecule can be placed in contact with the atom without penetrating any other atoms of the molecule'[4]. R is the sum of the Van der Waals radii of the atom and the solvent molecule.

In Fig. 1, the hydrophobicity of each of the 10 protein residue side chains is plotted against its accessible surface area. To derive the hydrophobicity of a side chain Nozaki and Tanford subtracted the value for glycine from that of the amino acid. I calculated the accessible surface areas of protein residue, X, from the accessibilities given by Lee and Richards[4] for X in tripeptide Ala-X-Ala, where the main chain is in an extended β conformation and the side torsion angles antiplanar. The values for the side chains in Fig. 1 are the accessible surface area of the residue minus the accessible surface area of glycyl (82 Å^2).

From Fig. 1 we see that for the non-polar side chains Ala, Val, Leu and Phe there is a linear relationship between hydrophobicity and accessible surface area; the correlation coefficient is 0.998 and the slope equivalent to 22 calorie Å^{-2}. For the side chains with hydroxyl groups Ser, Thr and Tyr there is a similar linear relationship with a correlation coefficient of 0.998 and a slope equivalent to 26 calorie Å^{-2}. For residues with similar accessible surface areas those with a polar group are about 1 kilocalorie less hydrophobic than those with only non-polar groups. In aqueous solution the OH, NH, N and S groups will form hydrogen bonds with water. But on transfer to ethanol or dioxane these solute-solvent hydrogen bonds are significantly weakened[5] and there is therefore a loss of free energy (1-2 kilocalorie per polar group is a reasonable estimate). In proteins this loss does not occur: ~ 92% of polar groups buried in the interior of proteins form hydrogen bonds. Thus the hydrophobicity of polar side chains will be greater than the values in Fig. 1. A 'correction' of 1–2 kilocalorie per polar group makes the polar residues as hydrophobic as non-polar residues. (The histidine side chain has two polar groups.) This implies that, if hydrogen-bonded, oxygen and nitrogen atoms are hydrophobic; they could, for example, be favourably accommodated in the interior of membranes. This result is related to the finding of Lee and Richards[4] that when lysozyme, RNase S and myoglobin go from an extended chain to the folded conformation, the reduction in accessible surface area is almost the same for polar and non-polar atoms. Methonine is a little less, and tryptophan a little more, hydrophobic than this argument and the linear relationship illustrated in Fig. 1, would suggest.

In conclusion, we can say that as a good approximation the hydrophobicity of residues in proteins is 24 calorie per Å^2 of accessible surface area. This value is applicable to protein folding, subunit contacts and the interaction of small molecules with enzymes and immunoglobins. For example the formation of the $\alpha_1\beta_1$ contact in haemoglobin reduces in each monomer the surface area accessible to water by ~900 Å^2 (my unpublished results). This implies that the hydrophobic contribution to the free energy of dimer formation is 43 kilocalorie. The formation of the contacts that cause the reduction in accessible surface area when proteins fold or aggregate involves large conformational entropy losses[6,7] and appreciable steric strain (Szabo and Karplus, personal communication) which substantially reduces the figure for the free energy.

The value given here for the hydrophobicity of protein surfaces confirms Kauzmann's hypotheses[1] that the entropic term that stabilises protein aggregation and the binding of small molecules is mainly a result of hydrophobic bonding, and suggests that only relatively small contact areas are necessary for stability. In haemoglobin ~12% of the ac-

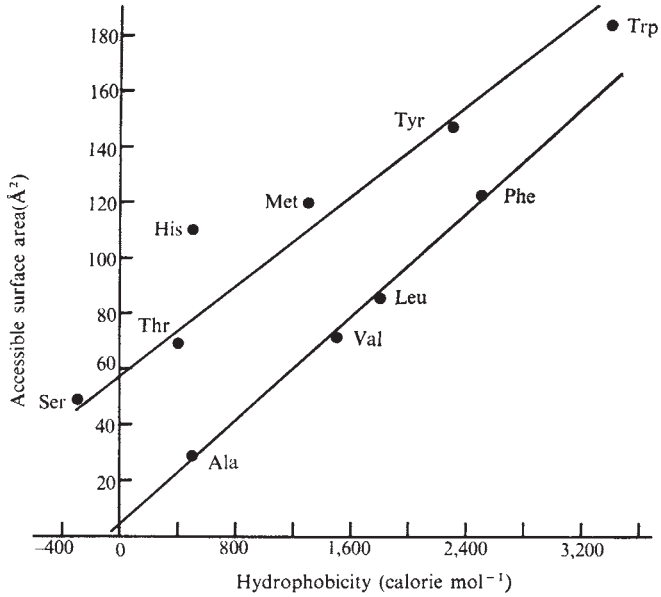


FIG. 1 Accessible surface areas of residue side chains (see text) plotted against hydrophobicity (free energy change for the transfer from 100% organic solvent to water[3]).

cessible surface area of each monomer is covered by the $\alpha_1\beta_1$ contact and ~6% by the $\alpha_1\beta_2$ contact.

In a discussion of the general theory of hydrophobic bonding Hermann[8] deduced and found a linear relationship between the logarithm of the solubility of hydrocarbons and the surface area of the cavity they form in water. This latter term is equivalent to the accessible surface area of the hydrocarbons.

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1 Kauzmann, W., *Advances in Protein Chemistry,* **14,** 1 (1959).
2 Klapper, M. H., *Progress in Biorganic Chemistry,* **2,** 55 (1973).
3 Nozaki, Y., and Tanford, C., *J. biol. Chem.,* **246,** 2211 (1971).
4 Lee, B., and Richards, F. M., *J. molec. Biol.,* **55,** 379 (1971).
5 Klotz, I. M., and Franzen, J. S., *J. Am. chem. Soc.,* **84,** 3461 (1962).
6 Page, M. I., and Jencks, W. P., *Proc. natn. Acad. Sci. U.S.A.,* **68,** 1678 (1971).
7 Kauzmann, W., in *The Mechanism of Enzyme Action* (Johns Hopkins University Press, Baltimore, 1954).
8 Hermann, R. B., *J. phys. Chem., Ithaca.,* **76,** 2754 (1972).

Insulin/proinsulin, a new crystalline complex

SINCE the first crystallisation of proinsulin in this laboratory[1], we have tried to grow crystals appropriate for detailed X-ray crystal structure analysis. None of the proinsulin crystal forms prepared proved ideal[2], and we attempted to cocrystallise insulin and proinsulin. Such crystals, if both components were in ordered array, could provide a basis for the determination of the structure of proinsulin by the use of rigid-body vector search methods[3,4] using the known stereochemistry of insulin[5].

Proinsulin is found in crystalline native insulin preparations[6]. It is common to find proteins from inhomogeneous fractions incorporated as impurities into crystals of the dominant component[7]. Very similar protein molecules, for example, β_1 and β_2 lactoglobulin[8] often form mixed crystals. These are defect solids, with all crystallographically equivalent positions occupied, but by molecules of different kinds. Steiner[9] has reported the preparation of what he seems to claim are mixed crystals of insulin and proinsulin of this type. Mixed crystals are not cocrystals if, by this we imply structures with each separate protein ordered by the full symmetry of the space group nor, because of disorder, are they useful for X-ray crystal structure analysis.

We report here the isolation and preliminary X-ray study of a 1:1 crystalline complex of insulin and proinsulin which seems to be a true cocrystalline form.

Cocrystallisation studies were made with both beef and pork hormone/prohormone pairs using the molar ratio 1:1 exclusively. Heterogeneous pairs were not studied. Separate insulin and proinsulin solutions of initial molarities 1.4 mM were prepared by dissolving each protein in 50 μl of a solution containing 0.05 M sodium citrate, 30 mg ml^{-1} sodium chloride, and, where used, 0.836 mg ml^{-1} of zinc chloride and 3 mg ml^{-1} of *m*-cresol. The final *p*H was adjusted to 7.25 by addition of 0.5 N HCl. Both proteins crystallise at this *p*H in the presence of *m*-cresol and zinc; insulin as diamond-shaped plates, proinsulin as tetragonal prisms with pyramidal termination. Hormone and prohormone solutions were warmed to 50° C with stirring, then mixed and kept at 50° C for a few minutes. Three vials of bovine material containing cresol and zinc were prepared. All gave a precipitate on cooling to room temperature. Protein-free crystallisation medium was added (with rewarming) to provide a series with final volumes of 130, 150 and 180 μl respectively. All vials were then kept at room temperature. Small, well-formed crystals began to appear after 2–4 d. The vials were then stored at 2° C. The crystals continued to grow for 1–2 weeks.

All preparations gave crystals. Only tiny, unidentified crystals, 50–60 μm long grew in the absence of *m*-cresol and/or zinc. The crystals in both pork and beef insulin—

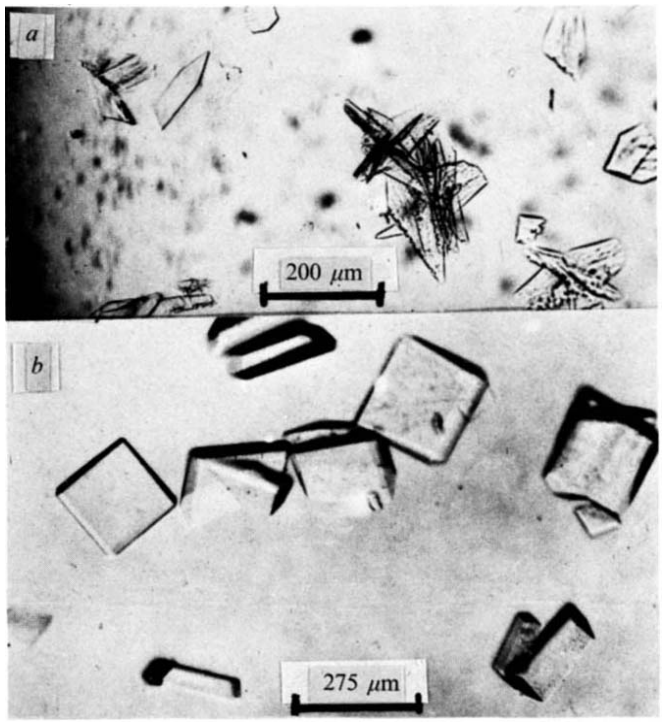


FIG. 1 *a*, 1:1 crystalline complex of porcine insulin/proinsulin; *b*, 1:1 crystalline complex of bovine insulin/proinsulin.

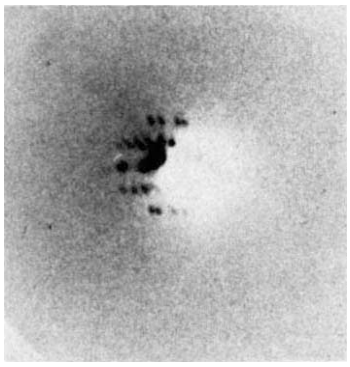


FIG. 2 Precession photograph of a bovine insulin/proinsulin complex, 72 h exposure ($\mu = 2.5°$).

proinsulin preparations are clear, colourless plates or wedges which extinguish sharply along the face diagonals under polarised light. The porcine crystals (Fig. 1*a*) were very thin, and less well formed than the bovine crystals which measured on average 190 μm × 190 μm × 30 μm (Fig. 1*b*).

Bovine insulin/proinsulin crystals were mounted in sealed thin-walled quartz capillaries and X-ray precession photographs taken using unfiltered Cuk_α ($\lambda = 1.5418$ Å) radiation from a Jarrel-Ash microfocus tube with a pinhole collimator. The crystals turned opaque after less than 30 h exposure to X rays at 20° C (crystals dissolve in their mother liquor at 21° C). Cooling to 15° C retards but does not halt radiation damage.

Useful diffraction patterns were obtained only with the X-ray beam approximately normal to the main face. From still photographs and two small angle ($\mu = 2.5°$) precession films (Fig. 2) the unit cell dimensions were determined to be 257 Å × 125 Å × 83 Å. It was not possible to determine the space group. The two longest dimensions lie along the face diagonals of the crystal.

The unit cell volume is 2.67×10^6 Å^3. The calculated weight fraction of protein based on a partial specific volume of 0.72 (ref. 1) for both insulin and proinsulin, and a crystal density of 1.20 g cm^{-3} (ref. 1) is 0.52. This is 10^6 daltons, equivalent to twelve hexamers each of insulin and proinsulin per unit cell.

The crystals were analysed by polyacrylamide disc gel electrophoresis, a procedure valid only if the crystals used are identical with the crystals studied by X-ray diffraction. After extensive microscopic survey the larger crystals were removed from those two vials used for X-ray study. These crystals (approximately 100) weighed about 10 μg. They were washed twice with separate 1 ml portions of protein-free buffer (that is, about 2.4×10^6 times their volume) to remove all non-crystalline protein on their surfaces.

The relative amounts of insulin and proinsulin in the crystals were then determined: The crystals were dissolved in Davis standard buffer (*p*H 6.7) and applied to gels prepared according to Davis' conditions[10]. Similar amounts of insulin and proinsulin were each applied to separate gels as positional standards. Gels were run for 50 min at 8 mA per gel, then stained with Buffalo Black NBR and scanned with a Canalco model E microdensitometer. The gel containing the dissolved crystals showed two bands at the positions of the insulin and proinsulin standards. The relative darkening of the two bands was identical, within 3%, to that on a standard gel run with an equimolar solution of insulin and proinsulin of approximately the same protein concentration.

To verify these findings we wished to use other methods of analysis. Amino acid analysis is insensitive and inappropriate because of the close similarity in amino acid composition between the two proteins. An alternative analytical procedure involving separation on a column and subsequent determination of insulin and proinsulin from measurement of absorbance was suggested to us by Steiner (personal communication, 1972) as more reliable than disc gel electrophoresis. It would unfortunately have required the preparation of prohibitively large amounts (~1 mg) of insulin/proinsulin crystalline material.

We finally chose to calibrate the protein-dye interaction using eleven solutions of insulin/proinsulin with molar ratios varying from 0.5 to 2.0 (1 μg μl^{-1} of protein). The plot of molar ratios of samples against the relative insulin/proinsulin band darkening effects is linear within the range studied and confirms our earlier finding. The insulin/proinsulin ratio is 1:1 within less than 5% maximum error.

We have prepared and characterised a 1:1 crystal complex with approximately twelve hexamers each of insulin and proinsulin per unit cell. As both proteins crystallise separately under the conditions used, there must be strong interactions leading to the new complex between insulin and proinsulin.

The crystals described here differ from any prepared by Steiner. The initial conditions for crystallisation are different (proinsulin has not been crystallised under the conditions used by Steiner). Steiner seems to imply that his crystals are mixed crystals (conforming, at least at low proinsulin ratios, to the dominant rhombohedral insulin form) containing one proinsulin molecule per eleven insulin molecules (8%) that is, on the average one proinsulin per two hexamers, or respectively one, two or three proinsulin molecules per hexamer.

We know of no studies which suggest that insulin and proinsulin form mixed dimers and hexamers in solution. It is difficult to accept the kinetics and equilibria required by the Steiner model—exchanges between homogeneous dimers and hexamers of insulin and proinsulin before crystallisation followed by prompt disassociation and complete reassociation into wholly insulin and proinsulin aggregates in the gel. The precise identity of the Steiner crystals must await adequate characterisation.

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1 Fullerton, W. W., Potter, R., and Low, B. W., *Proc. natn. Acad. Sci., U.S.A.*, **66**, 1213 (1970).
2 Rosen, L. S., Fullerton, W. W., and Low, B. W., *Archs. Biochem. Biophys.*, **152**, 569 (1972).
3 Nordman, C. E., and Nakatsu, K., *J. Am. chem. Soc.*, **85**, 353 (1963).
4 Nordman, C. E., *Acta crystallogr.*, **A28**, 134 (1972).
5 Adams, M. J., Blundell, T. L., Dodson, E. J., Dodson, G. G., Vijayan, M., Baker, E. N., Harding, M. W., Hodgkin, D. C., Rimmer, B., and Sheat, S., *Nature*, **224**, 491 (1969).
6 Chance, R. E., Ellis, R. M., and Bromer, W. M., *Science, N. Y.*, **161**, 165 (1968).
7 Aschaffenburg, R., and Drewry, J., *Biochem. J.*, **65**, 273 (1956).
8 Green, D. W., North, A. C. T., and Aschaffenburg, R., *Biochim. biophys. Acta*, **21**, 585 (1956).
9 Steiner, D. F., *Nature*, **243**, 528 (1973).
10 Davis, B. J., *Ann. N.Y. Acad. Sci.*, **121**, 404 (1964).

pH-dependent conformational change of gastrin

GASTRIN is a polypeptide hormone with a broad spectrum of biological activities, most notably the stimulation of secretion of gastric acid[1,2]. It is produced primarily in the pyloric gland area of the lower segment of the stomach, the antrum, which does not secrete acid[1,2]. When carried by the blood to the upper segment of the stomach, the fundus, gastrin stimulates secretion of acid by the oxyntic glands.[1] Although release of gastrin into the blood stream is mediated by stimuli from the nervous system, its release is strongly inhibited when the antral mucosa is bathed in acid[2]. Therefore, the secretion of the hormone is under negative feedback regulation, with resultant control of stomach acidity.

Human gastrin is a polypeptide composed of seventeen amino acid residues[3]:

1 5

Pyro Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-

10 15 17

Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2

It is found in two forms: without (gastrin I) and with (gastrin II) a sulphate esterified to the hydroxyl group of tyrosine-12. The amino terminus is blocked by the formation of a pyroglutamyl group, and the carboxyl-terminus is blocked as an amide. The carboxyl-terminal tetrapeptide amide fragment (residues 14 to 17) has the full range of physiological activities of the intact hormone[4]. No function for the amino-terminal tridecapeptide (residues 1 to 13) has been demonstrated. A striking structural feature of this portion of the hormone is the sequence of five glutamic acid residues (residues 6 to 10). Synthetic polymers of L-glutamic acid have been shown to undergo a transition from random coil to the α-helical conformation as the *p*H is lowered[5,6].

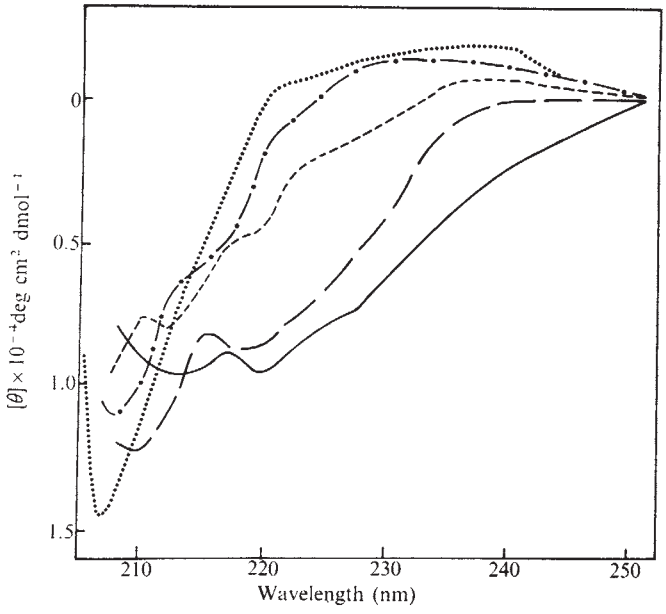


FIG. 1 Circular dichroic spectra of gastrin I at varying *p*H values. *p*H: ····, 8.10; —•—•, 6.68; – – – –, 4.85; — —, 3.91; ——, 2.81. Aliquots of a stock solution (1.0 mg ml^{-1} in 0.05 M NH_4HCO_3) were added to very dilute HCl solutions to give a gastrin concentration of 0.02 mg ml^{-1} at the indicated *p*H values. Circular dichroic spectra were measured with a Beckman CD spectrophotomer equipped with a Hewlett-Packard 5480A signal analyser. Measurements of samples in a 1.0 cm path length cell were made at 22°C. All spectra are averages of a minimum of 150 scans. Ellipticities ($[\theta]$) have been expressed in units of degree cm^2 per decimol of amino acid.

Since the antrum of the stomach is normally bathed in solutions with *p*H values in this same acidic region, it seemed possible that a similar *p*H-dependent conformational change of gastrin was involved in the inhibition of its release by acid. As a first step in testing this hypothesis, the circular dichroic spectra of gastrin as a function of *p*H were studied as probes of its conformations.

The circular dichroic spectra of synthetic human gastrin I (referred to here as gastrin, obtained from ICI, Cheshire,

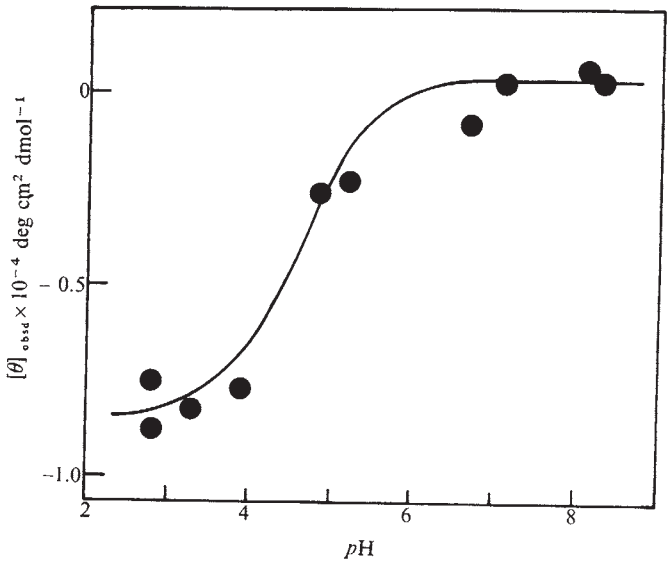


FIG. 2 Variation of the observed ellipticity of gastrin I at 222 nm with *p*H. Points were obtained experimentally. The curve is theoretical, and was calculated from equation (1).

England) at varying *p*H values is presented in Fig. 1. The spectrum measured near neutrality (for example at *p*H 8.10) has a small positive ellipticity in the 220 to 240 nm range and large negative ellipticity at shorter wavelengths. Qualitatively it is very similar to the spectrum of the random coil forms of poly-L-glutamic acid[7] and poly-L-lysine[7,8]. The circular dichroic spectrum of gastrin in acidic solution (for example at *p*H 2.81) has two peaks of negative ellipticity at 212 and 220 nm. These negative peaks are in approximately the same positions as the two peaks of negative ellipticity which characterise the α-helical conformations of poly-L-glutamic acid[7] and poly-L-lysine[7,8]. It is interesting that a maximum in negative ellipticity ($[\theta]$) centred near 217 nm which is characteristic of β-structure is absent from all spectra (Fig. 2), indicating the absence of substantial amounts of β-structure. Unfortunately, spectra recorded below 210 nm are not sufficiently free of noise to allow detection of an isosbectic point which would indicate the absence of a conformational intermediate between the random coil and α-helical forms. This low signal-to-noise ratio was a result of the low concentration of gastrin used, which was dictated by its low solubility in acidic solution.

The randow coil and β-structure have the same ellipticity at 222 nm[8]. Therefore, the magnitude of observed ellipticity $[\theta]_{obsd}$ at this wave length (all measurements and values of $[\theta]$ used below are at 222 nm) can be used to approximate the helical content of a protein or polypeptide at any given *p*H. The variation of $[\theta]_{obsd}$ with *p*H can be used as a measure of formation and breakdown of α-helical structures with change of *p*H. A plot of the observed ellipticity at 222 nm as a function of *p*H is presented in Fig. 2.

As a first approximation in attempting to derive a theoretical treatment to fit the dependence of $[\theta]_{obsd}$ on *p*H, two simplifying assumptions have been made. First, the conformational change and resultant change of $[\theta]_{obsd}$ depends on a single ionisation. Second, only the complete random coil, and the partial α-helical conformations may exist. The points in

Fig. 2 can be fitted to a theoretical curve described by equation (1), where $[\theta]_{obsd}$ is the

$$[\theta]_{obsd} = [\theta]_{\alpha\ obsd}\left[\frac{[H^+]}{K_{app} + [H^+]}\right] + [\theta]_{R+\beta\ obsd}\left[\frac{K_{app}}{K_{app} + [H^+]}\right] \quad (1)$$

observed ellipticity; $[\theta]_{\alpha\ obsd}$ is that fraction of the observed ellipticity attributable to α-helical structures, and $[\theta]_{R+\beta\ obsd}$ is that fraction attributable to random coil and β-structures. The terms $[H^+]/(K_{app} + [H^+])$ and $K_{app}/(K_{app} + [H^+])$ define the mol fraction of gastrin in the conformation which predominates under acidic or neutral conditions respectively. The curve shown in Fig. 3 has been calculated to give the best fit to the experimental points with the following values: $K_{app} = 2.51 \times 10^{-5}$ ($pK_{app} = 4.6$), $[\theta]_{R+\beta\ obsd} = +300$, and $[\theta]_{\alpha\ obsd} = -8{,}300$. The apparent pK_a is similar to that for the ionisation of a γ-carboxyl of a glutamic acid residue ($pK_a = 4.4$)[9]. The value of $[\theta]_{R+\beta\ obsd}$ is slightly more positive than $[\theta]_R = -2{,}780$ and $[\theta]_\beta = -2{,}670$ which have been derived from studies of five globular proteins[10], and within the limits of $[\theta]_R = +3{,}900$ and $[\theta]_\beta = -13{,}800$ found for poly-L-lysine[8]. Thus, the value of $[\theta]_{R+\beta\ obsd}$ is compatible with gastrin being a random coil in neutral solution. While the theoretical treatment of the *p*H-dependence of $[\theta]_{obsd}$ defined by equation (1) gives a good fit to the experimental data, it is probably an oversimplification. Additional factors may be considered which, if included, might give a more precise description of this helix to random coil transition. Their involvement is not obvious in the experimental data, however (Fig. 2).

As a first approximation the fraction of the gastrin molecule which assumes the α-helical conformation in acidic solution can be calculated from equation (2)[8],

$$\text{fraction } \alpha\text{-helix} = \frac{[\theta]_{\alpha\ obsd} - [\theta]_{R+\beta\ obsd}}{[\theta]_\alpha - [\theta]_{R+\beta\ obsd}} \quad (2)$$

where $[\theta]_\alpha$ is the ellipticity of a completely helical structure. If a value of $[\theta]_\alpha = -35{,}000$[8] is used, 0.24 of the gastrin molecule, or 4.1 amino acids, assume an α-helical conformation in acidic solution. If the value of $[\theta]_\alpha = -31{,}500$[10] is used, 0.27 of the gastrin molecule, or 4.6 residues, participate in the α-helix.

Equation (2) is based on the assumption that ellipticity ($[\theta]_\alpha$) is independent of the length of the helix. In fact, calculated circular dichroism spectra of polymers of alanine[11] indicated that $[\theta]_\alpha$ decreases with polypeptide chain length, most noticeably for short helical segments[10,11]. The simplest way to correct for this deviation is by a graphical method. If the ellipticity per decimol of helical segment (that is, $[\theta]_\alpha \times n$, where n is the number of amino acids per helical segment) is plotted as a function of the number of amino acids in the helix (n) a linear relationship is obtained (Fig. 3). If only one segment of helix is present in gastrin at low *p*H, then the ellipticity per decimol of gastrin (that is, $[\theta]_{\alpha\ obsd} \times 17 = 1.41 \times 10^4$) when included in this plot corresponds to $n = 5.2$ residues in an α-helix.

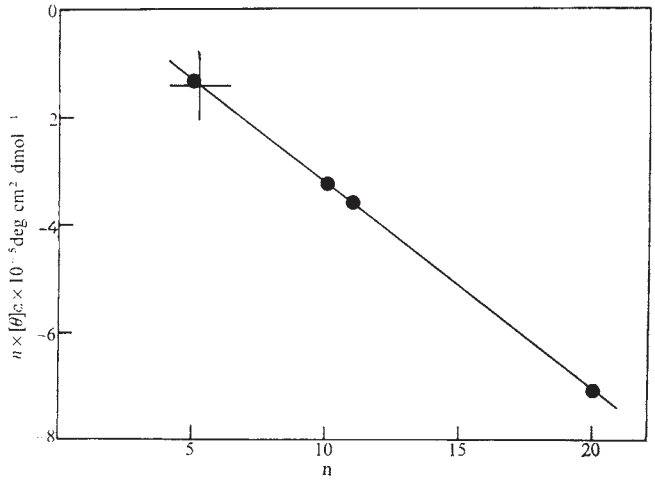


FIG. 3 Ellipticity per decimol of helical segment (that is, $[\theta]_\alpha \times$ the number of amino acids per helical segment) plotted as a function of the number of amino acids in the helix. Values of $n \times [\theta]_\alpha$ for 5, 10, and 20 residues were calculated from the ellipticities of polymers of alanine[10,11]; $n \times [\theta]_\alpha$ for 11 residues is an average value, calculated from the observed ellipticities of several globular proteins[11].

In view of the uncertainty involved in calculating by the above methods the number of residues which assume the helical conformation and the uncertainty inherent in the experimental data, it is reasonable to conclude that the α-helical structure of gastrin contains 5 ± 1 amino acid residues. Since polymers of L-glutamic acid are known to undergo a similar transition from random coil to α-helix, the amino acid residues of gastrin most likely to be involved in α-helix formation are the five sequential glutamic acids (6 to 10).

Gastrins from different species do not always contain the five consecutive glutamate residues. In gastrin from cat, cow and sheep, glutamate 10 is replaced by alanine[2], and in dog gastrin glutamate 8 is replaced by alanine[2]. Polymers of L-alanine have been studied extensively by X-ray diffraction and found to be in the α-helical form[12]. Also, on the basis of energy computations, alanine has been recognised as an amino acid which promotes α-helix formation[13]. Furthermore, in the tertiary structures of seven globular proteins, alanine has been found to have a greater than random probability of participating in an α-helix[14]. Thus, the substitution of alanine for glutamic acid in some species is not likely to prevent helix formation, although this has yet to be demonstrated.

Release of gastrin from the pyloric gland area of gastric mucosa of the stomach is inhibited when this tissue is bathed in acid[2]. If gastric acid could interact with gastrin within this tissue, the partial α-helical conformation would be induced. For any of a variety of possible reasons, the conversion of gastrin to the α-helical conformation might prevent its secretion. Thus, the *p*H-dependent conformational change of gastrin may play a physiological role in the mechanism of acid inhibition of its release.

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1 Grossman, M. I., *Fed. Proc.*, **27**, 1312 (1968).
2 Grossman, M. I., *Nature*, **228**, 1147 (1970).
3 Bentley, P. H., Kenner, G. W., and Sheppard, R. C., *Nature*, **209**, 583 (1966).
4 Morley, J. S., *Fed. Proc.*, **27**, 1314 (1968).
5 Doty, P., Wada, A. J., Yang, J. T., and Blout, E. R., *J. Poly. Sci.*, **23**, 851 (1957).
6 Fasman, G. D., in *Poly-α-Amino Acids* (edit. by Fasman, G. D.) 499 (Dekker, New York, 1967).
7 Holzwarth, G., and Doty, P., *J. Am. chem. Soc.*, **87**, 218 (1965).
8 Greenfield, N., and Fasman, G. D., *Biochemistry*, **8**, 4108 (1969).
9 Edsall, J. T., *Proteins, Amino Acids and Peptides* (edit. by Cohn, E. J., and Edsall, J. T.) 445 (Reinhold, New York, 1943).
10 Chen, Y.-H., Yang, J. T., and Martinez, H. M., *Biochemistry*, **11**, 4120 (1972).
11 Woody, R. W., and Tinoco, I., Jr., *J. chem. Phys.*, **46**, 4927 (1967).
12 Elliott, A., in *Poly-α-Amino Acids* (edit. by Fasman, G. D.) 1 (Dekker, New York, 1967).
13 Kotelchuck, D., and Scheraga, H. A., *Proc. natn. Acad. Sci. U.S.A.*, **62**, 14 (1969).
14 Crawford, J. L., Lipscomb, W. N., and Schellman, C. G., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 538 (1973)

Stimulation of calcitonin synthesis and release *in vitro* by calcium and dibutyryl cyclic AMP

It has been proposed that the physiological action of calcitonin is to prevent or diminish postprandial hypercalcaemia[1]. This is accomplished by inhibition of bone resorption[2], of the tubular reabsorption of calcium[3], and of the intestinal absorption of calcium[4]. Secretion or release of the hormone by parafollicular cells of the thyroid gland is mediated by calcium[5–7] and by gastrointestinal hormones including gastrin and pancreozymin[8,9]. Secretion is apparently influenced by the adenylate cyclase system [7,10] and hormonal regulation presumably is mediated by cyclic adenosine 3′, 5′-monophosphate (cyclic AMP).

We have carried out studies to determine whether calcium and dibutyryl cyclic AMP influence synthesis as well as release of calcitonin. For this purpose slices of porcine thyroid tissue were incubated for 3 h in Krebs-Ringer phosphate buffer. After incubation calcitonin in the incubation medium and in thyroid tissue extracts was determined by radioimmunoassay[7,11]. As compared with unincubated tissue, there was a three-fold and highly significant mean increase in total calcitonin when tissues were incubated in the presence of a high concentration of calcium, 5.0 meq l^{-1} (Table 1). Calcium, 7.5 meq l^{-1}, produced mean increases of 75% in the quantity of hormone released into the medium and of 58% in the total amount of hormone as compared to control samples incubated with no calcium in the medium (Table 2). Dibutyryl cyclic AMP, 10 mM, produced mean increases of 28% in the amount of calcitonin released into the medium and of 20% in the total quantity of hormone as compared with control samples incubated with no cyclic nucleotide in the medium (Table 3). These results, which are representative, show that thyroid slices readily synthesise calcitonin during a 3 h period of incubation and that calcium and dibutyryl cyclic AMP produce modest but significant mean increases in calcitonin synthesis as well as secretion.

Table 1 Effects of time and calcium* on synthesis and release of calcitonin *in vitro*.

Incubation (h)	Tissue	Calcitonin Medium (ng)	Total	No.
0	301 ± 46	—	301 ± 46	9
3	461 ± 80	752 ± 66	1213 ± 98	9
	NS		<0.001	

Slices of porcine thyroid glands weighing 100 mg (±5%) were used. Half of them were incubated in 4 ml Krebs-Ringer phosphate buffer, *p*H 7.4, for 3 h at 37° C under 100% O_2. Slices from a single gland were used in each experiment. Magnesium concentration was 3.1 meq l^{-1} and glucose concentration was 1 mg ml^{-1}. After incubation, the slices were freeze dried and extracted three times with butanol-acetic acid and water (75:7.5:21)[12]. The extracts were combined, freeze dried and taken up in the buffer used for the immunoassay[7]. The unincubated slices were similarly treated. Calcitonin in extracts and medium was estimated by radioimmunoassay in quadruplicate at two different dilutions[7,11].

Results are given as ng and are expressed as mean ± one s.e. Student's *t* test was used to determine statistical significance of differences.

* The incubation medium contained 5 meq l^{-1} of calcium.

Very little is known about the effects of long term stimulation by calcium on either the synthesis or secretion of calcitonin. Gittes *et al.*[13] showed that calcitonin content in thyroid glands of rats could be reduced by 65% with hypercalcaemia produced by the administration of calcium. Conversely, they demonstrated progressive accumulation of calcitonin content of the thyroid glands with hypocalcaemia produced by parathyroidectomy. Indeed, in the parathyroidectomised animals values of calcitonin content as high as twelve-fold that of values in control animals were found after 14 weeks. It was concluded that the thyroid gland is much better adapted to secrete calcitonin in response to acute hypercalcaemia than in response to chronic hypercalcaemia.

Table 2 Effects of calcium on the synthesis and release of calcitonin *in vitro*.

$[Ca^{2+}]$ (meq l^{-1})	Tissue	Calcitonin Medium (ng)	Total	No.
None	525 ± 79	724 ± 59	1249 ± 48	10
7.5	717 ± 70	1262 ± 178	1979 ± 168	10
	NS	< 0.01	< 0.001	

There is little information currently available concerning the effects and mechanism of action of cyclic AMP to stimulate the synthesis of protein hormones. In pulse-chase experiments, Tanese *et al.*[14] showed that the incorporation of ^{3}H-leucine into insulin and proinsulin and subsequent release of the hormone and prohormone by isolated islet cells of rats *in vitro* are increased by dibutyryl cyclic AMP. Similarly, Abe and Sherwood[15] demonstrated that incorporation of ^{14}C-leucine into parathyroid hormone and release by bovine parathyroid tissue *in vitro* is also augmented by dibutyryl cyclic AMP. These studies together with our own suggest that cyclic AMP may modify synthesis as well as secretion of protein hormones.

Table 3 Effects of dibutyryl cAMP on synthesis and release of calcitonin *in vitro*.

Dibutyryl cAMP (mM)	Tissue	Calcitonin Medium (ng)	Total	No.
None	531 ± 78	1255 ± 85	1786 ± 134	10
10	577 ± 84	1580 ± 81	2156 ± 108	10
	NS	< 0.02	< 0.05	

The concentration of calcium in the incubation medium was 2.5 meq l^{-1}.

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1 Gray, T. K., and Munson, P. L., *Science, N.Y.*, **166**, 512 (1969).
2 Friedman, J., and Raisz, L. G., *Science, N.Y.*, **150**, 1465 (1965).
3 Ardaillou, R., Vuagnat, P., Milhaud, G., and Richet, G., *Nephron*, **4**, 298 (1967).
4 Olson, E. B., DeLuca, H. F., and Potts, J. T., jun., *Endocrinology*, **90**, 151 (1972).
5 Care, A. D., Cooper, C. W., Duncan, T., and Orimo, H., *Endocrinology*, **83**, 161 (1968).
6 Lee, M. R., Deftos, L. J., and Potts, J. T., jun., *Endocrinology*, **84**, 36 (1969).
7 Bell, N. H., *J. clin. Invest.*, **49**, 1368 (1970).
8 Care, A. D., Bates, R. F. L., Swaminathan, R., and Ganguli P. C., *J. Endocr.*, **51**, 735 (1971).
9 Care, A. D., Bruce, J. B., Boelkins, J., Kenny, A. D., Conoway, H., and Anast, C., *Endocrinology*, **89**, 262 (1971).
10 Care, A. D., Bates, R. F. L., and Gitelman, H. J., *J. Endocr.*, **48**, 1 (1970).
11 Bell, N. H., *Nature new Biol.*, **245**, 85 (1973).
12 Gudmundsson, T. V., Byfield, P. G. H., Galente, L., and MacIntyre, I., in *Calcitonin: Proceedings of the Symposium on Thyrocalcitonin and the C Cells* (edit. by Taylor, S. F.), 51 (William Heinemann Medical Books Ltd., London, 1968).

[13] Gittes, R. F., Toverud, S. U., and Cooper, C. W., *Endocrinology*, **82**, 83 (1968).
[14] Tanese, T., Lazarus, N. R., Devrim, S., and Recant, L., *J. clin. Invest.*, **49**, 1394 (1970).
[15] Abe., M., and Sherwood, L. M., *Biochem. biophys. Res. Commun.*, **48**, 396 (1972).

Relationship between measles and canine distemper viruses determined by delayed type hypersensitivity reactions in dogs

MEASLES virus, canine distemper virus and rinderpest virus are classified as members of a subgroup of paramyxoviruses with various interrelationships (reviewed in ref. 1). In some species and in some combinations the viruses may provide a measure of protection against virulent heterologous challenge. This has been the premise for using measles virus vaccine to protect pups against canine distemper at an age when passive maternal immunity often interferes with canine distemper vaccination[2–5].

The principal protection against natural primary measles infection is considered to be cell-mediated immunity[6,7], and so it seemed likely that this mechanism also protects against canine distemper. Because of the relationship between the two viruses, we reasoned that measles virus should stimulate the response against distemper. We have tested this hypothesis with dogs to determine whether delayed hypersensitivity reactions could be elicited with both homologous and heterologous antigens.

We used the Edmonston strain of measles virus, which had been through twenty-four passages in human kidney culture, twenty-eight in human amnion culture, six in primary dog kidney culture and two in an established dog kidney cell line (NL-DK-1). The canine distemper virus used was the Snyder Hill strain which had been modified by twenty-eight passages in the NL-DK-1 cell line. The history and tissue culture procedures used for this cell line[8] and for the viruses[5] have been described previously. To prepare the sensitising antigens, a large sample of cells growing in Roux bottles was divided into three portions. One portion was infected with measles virus, another with canine distemper virus and the remainder was left uninfected. At the appropriate time, antigens were collected and frozen at −50° C. The live measles viral antigen had a virus titre of $10^{6.26}$ $TCID_{50}$ ml^{-1}, while that of the live canine distemper viral antigen was $10^{4.63}$ $TCID_{50}$ ml^{-1}.



FIG. 1 Delayed hypersensitivity reaction in ear of dog sensitised with measles virus. Perivascular infiltration of small lymphocytes and some polymorphonuclear leukocytes was seen 24 h after challenge with canine distemper viral antigen prepared as previously indicated. Haematoxylin and eosin stain, magnification ×224.

TABLE 1 Delayed hypersensitivity reaction in dogs sensitised with measles virus after challenge with canine distemper virus

Sensitising antigen	Challenge antigen: Canine distemper	Challenge antigen: NL-DK-1
Measles virus	3/4*	0/4
Canine distemper	2/2	0/2
NL-DK-1 (control)	0/2	0/2

* Numerator indicates number of positive reaction sites, denominator indicates number of dogs tested.

Cells used to prepare the skin test reagents were handled similarly except that after collection the virus was concentrated by differential centrifugation. Fluids were centrifuged at 2,000 r.p.m. in a 267A head in a model BE International centrifuge for 15 min followed by centrifugation at 55,000 r.p.m. in a SW 27 head on a model L3 Beckman centrifuge at 5° C for 120 min. After resuspension of the pellet in Hanks balanced salt solution, virus had been concentrated about forty-fold. The cell control antigen was handled in an identical manner. The resuspended concentrates were then treated with Tween-80 and ether as described by Norrby[9] which served to inactivate the viral antigens.

Our test procedure determined by preliminary experiments consisted of a single inoculation with a sensitising antigen (live virus) followed 10–30 d later by test inoculations with skin test reagents. The sensitising inoculations of 5.0 ml were given intramuscularly in the hind legs. Test inoculations of 0.2 ml were given subcutaneously in the inside surface of the ears. Up to three different antigens were inoculated at separate sites in each ear with inoculations being repeated bilaterally. Dogs had to be kept at a minimum temperature of 20° C, as at a lower temperature cold ears interfered with the test and gave false negative reactions. (This is important if experiments are conducted with animals maintained in the open.) Immediately before each inoculation, blood samples were tested for neutralising antibodies against both canine distemper and measles viruses[5]. Samples collected at the first inoculation were tested to determine that dogs had not been exposed previously. Samples collected at the second inoculation were tested to ensure that each dog had shown an appropriate response to the sensitising antigen alone. Dogs were examined 4, 24, 48, 72 and 96 h after subcutaneous challenge. No reactions indicative of an immediate hypersensitivity response were observed at 4 h. The first positive reactions, palpable at 24 h, consisted of a small region characterised by oedema and induration which had generally increased in size at 48 h. Lesions were not characterised by erythema. At 72 and 96 h the lesions were smaller but still palpable and gradually disappeared in about a week. In some experiments, dogs were rechallenged and the lesions tended to be larger and occasionally exhibited central necrosis.

TABLE 2 Delayed hypersensitivity reactions in dogs

Sensitising antigen	Challenge antigen: Canine distemper	Measles	NL-DK-1
Canine distemper	3/3*	2/3	0/3
Measles	3/3	3/3	0/3
NL-DK-1 (control)	0/2	0/2	0/2

* Numerator indicates number of positive reaction sites, denominator indicates number of dogs tested.

The purpose in our first experiment was to determine by comparison of lesions and histological sections whether sensitisation with measles virus would cause a dog to respond to a challenge with canine distemper antigen with a typical delayed hypersensitivity response (Table 1). Dogs sensitised with canine distemper virus or NL-DK-1 cells served as controls. Fourteen days later dogs were inoculated bilaterally with both canine distemper antigen and the NL-DK-1 cell antigen as control. After 24 h, under general anaesthesia, punch biopsies approximately 20 mm in diameter were removed at the sites of inoculation from one ear. After 48 h, similar biopsies were made on the other ear. Tissues were fixed in 10% neutralised formalin and stained with haematoxylin and eosin. The cell types seen in the lesions after 24 h were a mixture of mononuclear cells and poly morphonuclear leukocytes (Fig. 1). By 48 h the former were predominant. This response is similar to that reported for other antigens that induce delayed type hypersensitivity reactions in the dog[10].

In the second series of experiments, we extended the observations of delayed hypersensitivity responses to both homologous and heterologous antigens. One month after the first inoculation, test inoculations were made bilaterally on each ear with all three antigens. Table 2 shows that animals sensitised with canine distemper virus exhibited delayed hypensensitivity reactions when challenged with canine distemper or measles antigen. Animals sensitised with measles virus and challenged with both antigens also exhibited delayed hypersensitivity reactions. Sensitisation and challenge with preparations of NL-DK-1, which were uniformly negative, served as controls to assure that the reactions seen were not a result of response to components of the culture system. It is possible that common envelope antigens acquired from the membrane of the NL-DK-1 cells could be involved in cross reactions. This would be true if the acquired antigens were masked or inactive on the NL-DK-1 cells but were exposed and active when incorporated in the viral envelope.

So far no underlying immunological mechanism has been identified for heterotypic immunity, which is the veterinary term for the concept of using one virus to provide protection against another. This demonstration of delayed hypersensitivity to measles and canine distemper virus, and of cross reactions suggest that cell-mediated immune response is the mechanism by which measles vaccine protects pups against canine distemper virus.

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[1] Imagawa, D. T., *Prog. med. Virol.*, **10**, 160 (1968).
[2] Warren, J., Nadel, M. K., Slater, E. A., and Millian, S. J., *Am. J. vet. Res.*, **21**, 111 (1960).
[3] Slater, E. A., and Murdock, F. M., *Vet. Med.*, **58**, 717 (1963).
[4] Baker, J. A., Sheffy, B. E., Robson, D. S., and Gilmartin, J., *Cornell Vet.*, **56**, 588 (1966).
[5] Brown, A. L., Vitamvas, J. A., Merry, D. L., and Beckenhauer, W. H., *Am. J. vet. Res.*, **33**, 1447 (1972).
[6] Burnet, F. M., *Lancet*, ii, 610 (1968).
[7] Good, R. A., and Zak, S. J., *Am. Acad. Ped. Proc.*, **18**, 109 (1956).
[8] Brown, A. L., Davis, E. V., and Beckenhauer, W. H., *Cornell Vet.*, **58**, 593 (1968).
[9] Norrby, E., Proc. *Soc. expl. biol. Med.*, **111**, 814 (1962).
[10] Streilein, J. W., and Barker, C. F., *J. Immun.*, **98**, 601 (1967).

Natural resistance to *Salmonella* infection, delayed hypersensitivity and Ir genes in different strains of mice

THE development of immunity to an intracellular bacterial infection such as that produced by *Salmonella typhimurium* in mice can be followed by the fall in the numbers of living bacteria in the liver and spleen. Resistance to infection appears at about the same time as delayed hypersensitivity to appropriate antigens of the infecting organisms and both may be taken as indicators of a cell-mediated immune response[1]. Inbred mouse strains differ widely in their resistance to salmonella infections and one possible explanation is that they differ in their ability to produce cell-mediated immune responses in general. This has been tested by comparing the degrees of delayed hypersensitivity detectable in different strains of mice sensitised with a number of unrelated antigens. The results discussed here disprove the hypothesis but suggest a more interesting one.

Six strains of inbred mice of differing ancestry[2] were taken and the LD_{50} for each, calculated after subcutaneous infection with *S. typhimurium* C5 (Fig. 1). The mouse strains could easily be separated into those sensitive to less than 100 organisms and those resistant to over 10^5. No intermediate grades of resistance were found.

An extract was prepared from a 48 h culture of *S. typhimurium* by an adaptation of the method of Collins and Mackaness[3] and the final protein concentration adjusted to 1 mg ml^{-1}. Mice infected with *S. typhimurium* 8 d previously were challenged by the injection of 0.025 ml of extract into the right hind footpad. The left hind footpad was injected with phosphate-buffered saline, and similar injections were made in unsensitised mice. Other mice of each strain were sensitised with BCG, picryl chloride and oxazolone. Mice

Mouse strain	LD_{50}
Balb/c	<10
C57Bl	2.10
B10.D2(new line)	5.10
DBA/2	2.10^5
C3H/He	1.10^6
A/JAX	2.10^6
CBA	1.10^7

FIG. 1 LD_{50} of inbred mouse strains after subcutaneous injection of *Salmonella typhimurium* strain C5.

TABLE 1 Delayed hypersensitivity reactions of the different mouse strains to all the sensitising agents

Sensitising agent	Sensitive strains Balb/c	C 57Bl	B10.D2	Increases*	Resistant strains DBA/2	C3H	A/JAX	CBA	Increases*
S. typhimurium									
× 10 sens. × 10^3 res. C5	0.08	0.03	0.12	0.08	0.18	0.20	0.29	0.24	0.23†
× 10 sens. × 10 res. C5	0.08	0.03	0.12	0.08	0.14	0.13	0.12	0.26	0.16‡
× 10^4 sens. × 10^4 res. M525	0.09	0.25	0.12	0.15	—	0.31	0.27	0.44	0.34†
Picryl chloride	0.08	0.05	0.05	0.06	0.05	0.06	0.11	0.09	0.08§
Oxazolone	0.07	0.11	0.18	0.12	0.12	0.09	0.11	0.12	0.11§
BCG	0.13	0.36	0.27	0.25	—	0.18	0.12	0.32	0.21§

* Increased thickness in mm of footpads (*S. typhimurium* and BCG experiments) or ears (picryl chloride and oxazolone experiments). Corrected means of 24 h and 48 h values. Comparing resistant and sensitive strains,† $P < 0.01$; ‡, $P < 0.05$; §, not significant.

were sensitised to BCG by intravenous injection of 10^5 live organisms and challenged 5 weeks later with 0.025 ml of tuberculin purified protein derivative (PPD) (10 μg ml^{-1}). Picryl chloride and oxazolone were applied to the clipped backs of the mice as 0.1 ml of a 3% solution in alcohol. Mice were challenged after 7 d with 2 drops of a 1% solution in olive oil spread on the right ear lobe, using pure olive oil as a control on the left.

Increases in thickness of footpads and ears were measured by a micrometer 4, 24 and 48 h after challenge. The immediate reaction subsided at 6 h and was followed by an increased reaction reaching a maximum at 24–48 h. This was accompanied by a mononuclear cell infiltration into the area, typical of a delayed hypersensitivity reaction. All the results have been corrected for the increases found in sensitised and unsensitised controls.

In the first series of experiments the sensitive strains were sensitised with ten *S. typhimurium* organisms and the resistant with 10^3. Although the resistant strains clearly developed an increased delayed hypersensitivity reaction to the extract, this could conceivably have been a result of the larger sensitising dose they had been given. All the mouse strains were therefore sensitised with a low dose of *S. typhimurium*. The resistant strains still gave a significantly greater reaction, which was confirmed by a similar experiment in which all strains of mice were given a high sensitising dose of the avirulent strain of *S. typhimurium*, M525, (Fig. 2*a*). In contrast, the responses to BCG, picryl chloride and oxazolone (Fig. 2*b*) were similar in all strains of mice (Table 1). Strains of mice naturally resistant to *S. typhimurium* infection therefore show a greater capacity than sensitive strains to develop delayed hypersensitivity reactions to *S. typhimurium*. This is, however, a specific reaction to a salmonella antigen or antigens and not a general capacity to develop good cell-mediated immunity. This finding is in agreement with that of Robson and Vas[3], who were able to immunise their resistant more readily than their susceptible mouse strains, using a variety of salmonella antigens. They suggested that the immunity produced was cell mediated and pointed out that the response to other organisms such as *Listeria monocytogens and Cryptococcus neoformans* did not run parallel.

The differences in resistance to salmonella infection of the inbred strains of mice must clearly have a genetic basis. The polarisation into strains of high and of low resistance with the absence of intermediate strains suggests that only a small number of genes, or even only one, is involved. Here our results differ from those of Robson and Vas who found that C3H/HeJ strains were susceptible and DBA/2J were intermediate. Both they and we used *S. typhimurium C5* (obtained from Professor Rowley) as the challenge organism. The difference in results may well be due to the different routes of infection used, intraperitoneal by Robson and Vas, subcutaneous in our present work. Mice infected intraperitoneally are frequently more susceptible than when infected by the subcutaneous or intravenous routes. In the poorly protected peritoneal cavity, bacteria have a chance to grow up; effectively increasing the inoculum[4,5]. This is supported by the relatively low doses of bacteria which killed A/J mice in Robson and Vas's experiments compared with ours. Precise comparison of results is made difficult because we have expressed resistance in terms of LD_{50}, whereas Robson and Vas have measured prolongation of survival time.

Comparison of the results presented here with those of Levine[6] and of Vaz *et al.*[7] shows that the resistant strains A, CBA and C3H are those which readily produced antibodies to low doses of a benzylpenicilloyl-bovine gamma globulin complex (BPO-BGG) or of ovomucoid but gave little or no response to ovalbumin. In contrast the susceptible strains C57Bl, Balb/c and B10-D2 responded poorly to BPO-BGG and ovomucoid but well to ovalbumin. DBA/2 mice which were the least resistant of the resistant group are anomalous in that although they responded to the salmonella extract they did not respond to BPO-BGG or to ovomucoid and relatively poorly to ovalbumin. Moreover, when sensitised with large

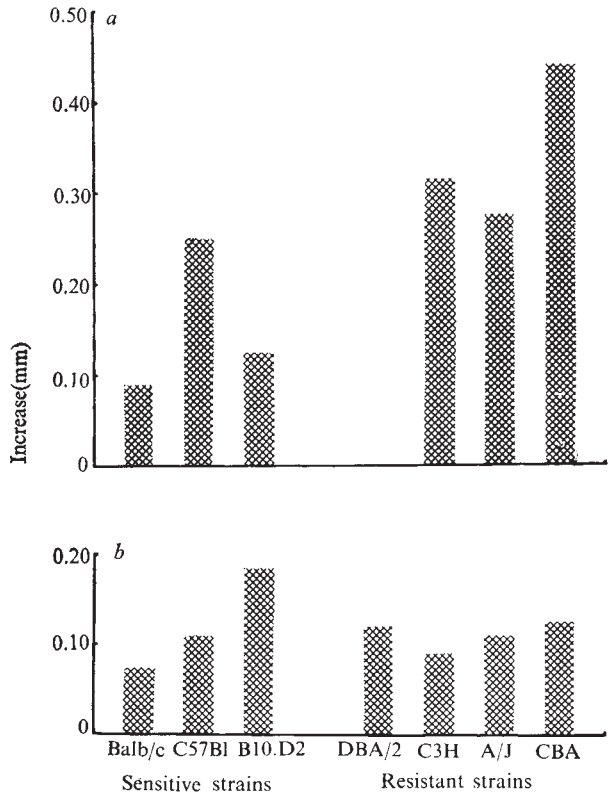


FIG. 2 *a*, Delayed hypersensitivity reactions to salmonella extract in mouse strains sensitised with 10^4 *S. typhimurium* strain M525. *b*, Delayed hypersensitivity to oxazolone. Increased thicknesses in mm of footpads (*a*) and ears (*b*) are the corrected means of the 24 h and 48 h values.

doses of the avirulent strain of *S. typhimurium* M525 they invariably died within 4 h of being challenged with the salmonella extract, apparently with some acute hypersensitivity reaction. Delayed hypersensitivity could not therefore be measured in these experiments.

Production of antibody to ovomucoid, ovalbumin and BPO-BGG in mice is thought to be controlled by immune response (Ir) genes[6–9] and these are closely, though perhaps not invariably, associated with histocompatibility genes. In the data so far, the resistant strains A/J are H2a, CBA and C3H are H2k, of the susceptible strains, C57Bl are H2b and Balb/c H2d. DBA/2 is again odd in being resistant, but H2d. Ir genes are generally dominant. Preliminary experiments with the F_1 generation of a Balb/c, CBA cross show that resistance is also dominant. Further experiments with backcrosses to check the relations between resistance and antibody responses to ovomucoid and ovalbumin and H2 types are in progress. At present, we suggest that a major factor in the resistance of mice to salmonella is the presence of an Ir gene controlling the response to some unknown but probably protein antigen present in *S. typhimurium*. Further characterisation of this antigen is required. In the extensive literature[10,11] on the basis of genetic resistance to salmonella infections in mice, many effector mechanisms such as antibody production, cellular immunity, phagocytic activity and macrophage bactericidal capacity have been considered. Current work on Ir genes using synthetic and other antigens may help solve the problems.

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1 Collins, P. M., and Mackaness, G. B., *J. Immun.*, **101**, 830 (1969).
2 Staets, J., *Biology of the Laboratory Mouse*, second ed., 1 (McGraw Hill, New York, 1966).
3 Robson, H. G., and Vas, S. I., *J. infect. Dis.*, **126**, 378 (1972).
4 Blanden, R. V., Mackaness, G. B., and Collins, F. M., *J. exp. Med.*, **124**, 583 (1966).
5 Collins, F. M., *J. Bact.* **97**, 667 (1969).
6 Levine, B. B., *Biochemistry of the Acute Allergic Reactions* (edit. by Austen, K. F., and Becker, E. L.) 1 (Blackwell, Oxford, 1971).
7 Vaz, N. M., Phillips-Quagliata, J. M., Levine, B. B., and Vaz, E. M., *J. exp. Med.*, **134**, 1335 (1971).
8 McDevitt, H. O., and Chinitz, A., *Science*, **163**, 1207 (1969).
9 Martin, W. J., Maurer, P. H., and Benacerraf, B., *J. Immun.*, **107**, 715 (1971).
10 Gowen, J. W., *A. Rev. Microbiol.*, **2**, 215 (1948).
11 Boehme, D. H., *Bull. N.Y. Acad. Med.*, **46**, 499 (1970).

Specific degranulation of human polymorphonuclear leukocytes

The human polymorphonuclear leukocyte (PMN) contains two types of cytoplasmic granules, specific or heterophil granules and azurophil granules[1]. These granules contain enzymes and various non-enzymatic proteins. The specific granule contains alkaline phosphatase and most of the lysozyme of the cell while the azurophil granule contains the acid hydrolases and other enzymes which identify it as a lysosome[1]. Recent work[2] has shown that in the degranulation accompanying phagocytosis the specific or heterophil granules combine with the phagosome first, followed by the azurophil granules. This implies that degranulation may be a complex event with more than one signal involved. If this were true then one might expect that the events should be separable, that is, that one type of granule might be stimulated and the other little affected. Experiments in our laboratories have indicated that only one type of PMN granule responds to phorbol myristate acetate (PMA). Five minutes after application of nanogramme quantities of the agent the cell begins to show large intracellular vacuoles. Granules which demonstrate an alkaline phosphatase reaction disappear in 30 min while those staining for myeloperoxidase remain[3]. Here we report that PMA causes both a dose and a time dependent release of enzyme activity associated with specific granules while those associated with azurophil granules or lysosomes remain associated with the cell as did a cytoplasmic marker enzyme glucose-6-phosphate dehydrogenase.

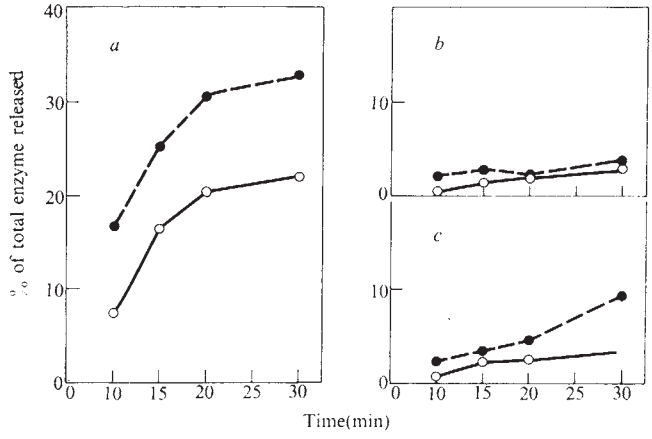


Fig. 1 *a*, Lysozyme; *b*, myeloperoxidase; *c*, β-glucuronidase. PMN (3×10^7) were incubated with or without PMA added at 0 time. The percentage of total enzyme released into the HBSS solution is indicated and in each case a time-matched control without PMA was subtracted from the total enzyme released. o, 5 ng ml^{-1} PMA; •, 20 ng ml^{-1} PMA.

The human PMN used in these studies were all sedimented through dextran and washed as previously described[4]. Red blood cells were lysed by 0.87% ammonium chloride[5]. The cells in these preparations were approximately 85% PMN, the remainder comprising lymphocytes, monocytes and platelets. These cells were incubated in a CO_2 incubator in Hanks Balanced Salt Solution (HBSS) without phenol red or serum. At the end of each incubation the cells were sedimented at 250 *g* and the supernatant was used for determination of enzyme activity released from the cells. The cell pellet was resuspended in 0.2 M sucrose plus 0.1% Triton X100 and then quick frozen (acetone-dry ice) and thawed five times. The enzyme activity in this material was defined as cell associated. Addition of this activity and that released from the cells is defined as total enzyme activity. The *p*H of the supernatant was not altered by the addition of PMA. The addition of the supernatant to the enzyme reaction mixtures described below did not alter the *p*H of these mixtures. The procedures for assay of lysozyme, myeloperoxidase, glucose-6-phosphate dehydrogenase[6], β-glucuronidase[7] and alkaline phosphatase[8] are described elsewhere. In each experiment, illustrated in Table 1 and Fig. 1, assay of cell-associated enzymes in the presence and in the absence of PMA indicated that PMA did not inhibit or enhance the activity of any of the enzymes tested. The cells were not permeable to trypan blue either at the start or end of each experiment. In addition, the cells did not lyse during the incubation, since they fail to release either the cytoplasmic enzyme (glucose-6-phosphate dehydrogenase; not shown) or enzymes of the azurophil granule.

TABLE 1 Effect of PMA on the release of enzymes from polymorphonuclear leukocytes

	Incubation time (min)	Percentage of total enzyme released* Lysozyme	Alkaline phosphatase	Glucuronidase
Control	15	4.7	–	7.1
5 ng ml^{-1} PMA	15	21.4	29.5	6.4
20 ng ml^{-1} PMA	15	31.5	41.0	5.7
20 ng ml^{-1} PMA	5	7.3	10.4	3.2

* Calculated from loss of cell-associated enzyme during incubation of PMN with PMA, compared with cell-associated enzyme at 0 time and cell-associated enzyme of matched control incubated in the absence of PMA. The presence of phosphate buffer in the medium (HBSS) precludes the determination of alkaline phosphatase activity in the medium. Alkaline phosphatase activity released in this table therefore represents the difference between total enzyme activity of control and experimental values.

PMA was obtained from Consolidated Midland Corp. Brewster New York. Each lot was examined by a mass spectrometer (showed a single molecular weight of 616) and by thin layer chromatography (migrated as a single spot).

Table 1 and Fig. 1 illustrate the effects of PMA on the release of enzyme from the PMN. Table 1 shows that both lysozyme and alkaline phosphatase responded similarly to comparable concentrations of PMA. In this experiment the amount of β-glucuronidase released was similar in control cells and cells exposed to PMA. Figure 1 shows the time course of release of lysozyme, myeloperoxidase and β-glucuronidase after treatment with 5 and 20 ng ml^{-1} PMA. In this experiment all enzyme activity shows an increase which is dependent on both PMA concentration and time of exposure. However the enzymatic activity represented in the specific granule (lysozyme) rises sharply and early. The lines in the graph are not carried through the origin since little or no release of enzyme activity is detectable in the 5 min after addition of PMA (see Table 1). In all of these experiments the cytoplasmic enzyme glucose-6-phosphate dehydrogenase did not appear in the external media.

Our data indicate that PMA causes the external degranulation of the PMN specific granule. Enzyme activity associated with this organelle is either present in the media and (or) lost from the cells after exposure to PMA. This event depends both on dose and time. Enzyme activities associated with azurophil granules remain almost entirely associated with the cell. These findings are consistent with observations that the alkaline phosphatase reaction is absent in cells exposed to PMA while large myeloperoxidase-negative vacuoles arise within the cells[8]. In addition myeloperoxidase-staining organelles continue to be present in cells containing many vacuoles[8]. This is the first report of external degranulation of specific granules alone. One of the early effects of PMA is to cause a rapid rise in intracellular cyclic GMP concentration in both 3T3 fibroblasts and human platelets[9]. Further, our previous experiments[10] have implicated cyclic GMP as the intracellular mediator of the action of PMA on the PMN to enhance chemotaxis. Other workers have also indicated that external degranulation of PMN which involves both specific and azurophil granules[11] may be enhanced by agents which increase intracellular cyclic GMP and by cyclic GMP itself[12,13]. This suggests that PMA causes external degranulation by a mechanism which may be initiated or modulated by an increase in cyclic GMP concentration. As Bainton[2] has demonstrated, however, internal degranulation involves both types of granular organelles in sequence; first the specific granule and then the azurophil granule rapidly fuse with the phagosome. A similar sequential mechanism probably also operates in external degranulation. If this is the case, then exposure to PMA results in the initial half of the event, fusion of the specific granule with the external cell membrane. Subsequent events leading to fusion of azurophil granules with the membrane do not occur. Our oservations, then, combined with those of Bainton[2] suggest that two signals may exist for degranulation.

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1 Bainton, D. F., and Farquhar, M. G., *J. Cell Biol.*, **39**, 286 (1968).
2 Bainton, D. F., *J. Cell Biol.*, **58**, 249 (1973).
3 White, J. G., and Estensen, R. D., *Am. J. Path.* (in the press).
4 Holmes, B., Page, A. R., and Good, R. A., *J. clin. Invest.*, **46**, 1422 (1967).
5 Baehner, R. L., and Karnovsky, M. L., *Science*, **162**, 1277 (1968).
6 Holmes, B., Day, N., Haseman, J., and Good, R. A., *Infect. Immun.*, **5**, 232 (1972).
7 Fruton, J. S., *J. biol. Chem.*, **166**, 721 (1946).
8 Hirschhorn, R., Hirschhorn, K., and Weissman, G., *Blood*, **30**, 84 (1964).
9 Goldberg, N. D., Haddox, M. K., Estensen, R. D., White, J. G., Lopez, C., and Hadden, J. W., in *Cyclic AMP in Immune Response and Tumor Growth* (edit. by Lichtenstein, L., and Parker, C.) (Springer-Verlag, in the press).
10 Estensen, R. D., Hill, H. R., Quie, P. G., Hogan, N., and Goldberg, N. D., *Nature*, **245**, 458 (1973).
11 Henson, P. M., and Oades, Z. G., *J. Immun.*, **110**, 290 (1973).
12 Columbo, C., Ignarro, L. J., and Chant, J. K., *Fedn Proc.*, **32**, 291 (1973).
13 Zurrier, R. B., Tynan, N., and Weissmann, G., *Fedn. Proc.*, **32**, 744 (1973).

Calcium does not uncouple oxidative phosphorylation in tightly-coupled mitochondria from Ehrlich ascites tumour cells

ENERGY-linked Ca^{2+} accumulation occurs in mitochondria isolated from different tissues and species (for reviews see refs 1 and 2). If the Ca^{2+} concentrations are kept relatively low, such accumulation does not lead to any apparent biochemical damage to the mitochondria, particularly to those processes associated with ATP synthesis. Once the concentration of Ca^{2+} in the incubation system approaches values of 100–150 nmol per mg protein, however, the ion induces mitochondrial swelling, release of the accumulated Ca^{2+} and total loss of acceptor control of respiration[3–6]. The latter situation is brought about through an increase in state IV respiration to a rate which approaches that of state III.

Other events which occur include stimulation of ATPase activity, greatly diminished ADP-ATP exchange and $^{32}P_i$-ATP exchange activities and P/O ratios[4,6]. All these events reach maximum at approximately identical concentrations of Ca^{2+} and so seem to reflect some common interaction of this ion at the intact (inner) membrane with which these energy-linked reactions are intimately associated.

On studying the interaction of Ca^{2+} with tightly coupled mitochondria isolated from Ehrlich ascites tumour cells (refs 7, 8 and R. F. W. T. and F. L. B., submitted for publication) these organelles showed membrane-linked responses to Ca^{2+} quite uncharacteristic of those seen in mitochondria isolated from most normal mammalian tissues, for example rat liver. The most conspicuous uncharacteristic response in these tumour mitochondria was the inability of Ca^{2+} to uncouple phosphorylation from respiration. The mitochondria are highly tolerant to exceptionally high concentrations of Ca^{2+}. Consequently, membrane-bound, energy-linked reactions normally grossly modified in the presence of 100–150 nmol Ca^{2+} per mg protein are either insensitive to or only slightly modified by concentrations as high as 300 nmol per mg protein. This finding suggests an altered metabolic behaviour in some tumour cells.

Mitochondria were isolated from a hyperdiploid strain of Ehrlich ascites tumour cells, by methods described elsewhere[7,8]. EDTA was present in the original isolation medium but was omitted from the medium used for the final washings of the mitochondria. The acceptor control of respiration and oxidative phosphorylation were both measured as described in the legends accompanying the appropriate figures. Oxygen uptake was measured using an oxygen electrode coupled to a Rikadenki pen recorder.

ADP-ATP and $^{32}P_i$-ATP exchange activities were measured as previously described[9], with the exception that in the case of the ADP-ATP exchange reaction the adenine nucleotides were separated by DEAE-cellulose paper chromatography[10]. ATPase activity was monitored by measuring the appearance of P_i in the medium using the method of Baginski *et al.*[11]

Sonication of the mitochondria was carried out using six 15-s intermittent exposures on an MSE 100 W sonicator, using a small probe. The suspension of mitochondria was maintained on ice during this period. The acceptor control ratio after sonication was one.

Ca^{2+} accumulation by mitochondria was measured using $^{45}Ca^{2+}$; Millipore filters (pore size 0.45 μm) were used to separate the mitochondria from the suspending medium.

The reagents used were all of AR grade. Adenine nucleotides were obtained from C. F. Boehringer and Sonne, Mannheim, Germany. Radioactive $^{45}Ca^{2+}$ was obtained from the Radiochemical Centre, Amersham, England. $^{32}P_i$ was obtained from Lucas Heights, New South Wales, Australia.

The traces shown in the inset to Fig. 1 illustrate the influence of high calcium concentration on the rate of respiration before (state IV) and after (state III) addition of ADP. Trace *a* indicates that ADP stimulated respiration some 3.5-fold in the absence of added Ca^{2+}. The amount of ADP added was such that state III respiration proceeded to anaerobiosis.

Trace *b* was obtained with an identical incubation medium except that Ca^{2+} (660 nmol) was added to the system before the addition of mitochondria. With the particular sequence of additions made in these experiments (Ca^{2+} before mitochondria) it is not possible to measure quantitatively the amount of extra oxygen taken up by the mitochondria due to the Ca^{2+} present in the medium. The rate of oxygen uptake was, however, qualitatively greater for a short period immediately following the addition of mitochondria; this increased rate would reflect the stimulation by Ca^{2+} of state IV respiration in these mitochondria as reported elsewhere (ref. 7, and submitted for publication). A most unusual feature of the trace is that despite accumulation and retention of this large amount of Ca^{2+} by the mitochondria (submitted for publication) (and see below), the rate of respiration after accumulation was complete and did not increase significantly as it does with rat liver mitochondria[1,4,7]. Also addition of ADP had no effect on the rate of respiration. These experiments are described in more detail elsewhere (submitted for publication).

The remaining data in Fig. 1 were obtained from traces similar to those described above but over a wide range of Ca^{2+} concentrations. The ADP-stimulated rate of respiration gradually declines to approach the same as that in the absence of ADP at about 250 nmol per mg protein. The acceptor control ratio which is maximal in the absence of added Ca^{2+} also gradually declines to a value approaching 1 at the same high level of Ca^{2+}.

Three features of these data thus contrast with the events that are seen under identical experimental conditions with mammalian mitochondria such as those isolated from rat liver (compare with data from ref. 4): (a) the ability to accumulate and retain high levels of Ca^{2+}, (b) the complete lack of an increase in the resting rate of state IV respiration once the high levels of Ca^{2+} have been accumulated by the mitochondria and (c) the inhibition of succinate-supported state III respiration.

Although the acceptor control ratios obtained with both rat liver and the ascites tumour mitochondria each approach a value of unity as the concentration of Ca^{2+} in the medium is increased (contrast data in ref. 4), it is important to note that each does so for entirely different reasons. With the liver mitochondria the value approaches unity because the rate of state IV respiration increases to that of state III.

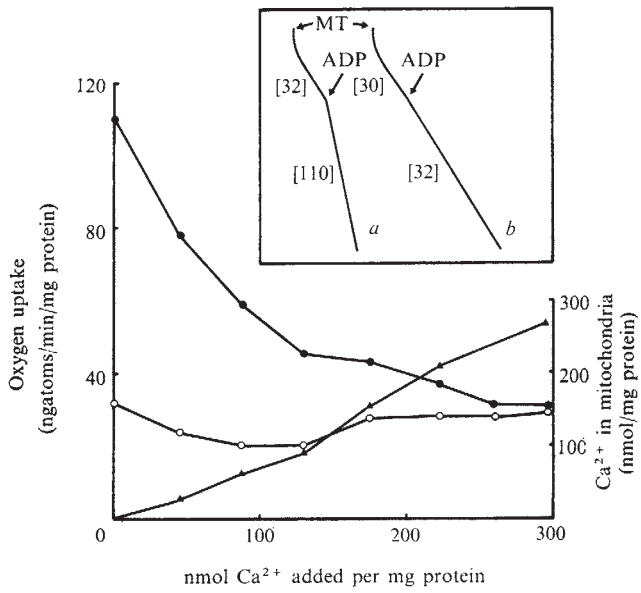


FIG. 1 Influence of Ca^{2+} concentrations on states III and IV of respiration in intact mitochondria isolated from Ehrlich ascites tumour cells. The incubation system contained (in order of addition) 80 mM sucrose, 16 mM Tris-HCl (*p*H 7.4), 1 mM (K) phosphate, 7 mM succinate, increasing concentrations of Ca^{2+} (containing $^{45}Ca^{2+}$) as indicated and 2.2 mg of mitochondrial protein. The total volume was 1.5 ml and the reaction temperature 25° C. Oxygen uptake in state IV (○) was measured for approximately 2 min before the addition of 1 mM ADP. When the state III trace (●) approached anaerobiosis (usually approximately 4 min after addition of ADP) a sample of medium was removed and filtered (Millipore; pore size, 0.45 μm) and the mitochondria analysed for the presence of $^{45}Ca^{2+}$ (▲). Numbers in brackets in the inset are the rates of O_2 uptake in the absence (*a*) and presence (*b*) of 660 nmol Ca^{2+}.

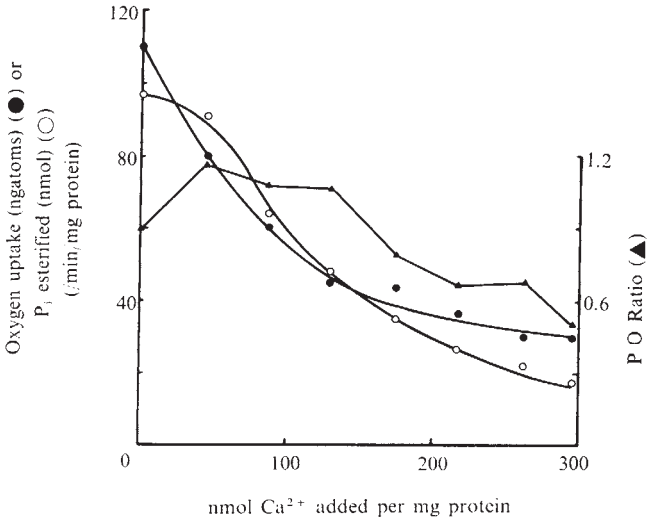


FIG. 2 Influence of Ca^{2+} concentration on the P/O ratio in intact mitochondria from Ehrlich ascites tumour cells. The incubation system was identical to that described in Fig. 1 except that $^{45}Ca^{2+}$ was not added. Also, phosphate (containing approximately 0.5×10^6 c.p.m. $^{32}P_i$) was added with the ADP. An aliquot of the reaction medium was removed into perchloric acid for assay of esterified $^{32}P_i$ at a known time, approximately 3 min, after addition of the ADP plus $^{32}P_i$. The P/O ratios were calculated from the total oxygen uptake from the time of the combined addition of ADP and $^{32}P_i$ until the reaction was stopped in perchloric acid.

With the tumour mitochondria, however, state III respiration declines to approach that of state IV.

The unusual respiratory behaviour of the tumour mitochondria induced by Ca^{2+} as described above prompted an examination of the effect of this ion on the P/O ratio. Data from one such experiment are shown in Fig. 2. The curve relating phosphate esterification to Ca^{2+} concentration shows only a gradual decline over the entire range of Ca^{2+} concentrations examined. At no stage is there any sign of the marked response of this function to Ca^{2+} as seen for example in rat liver mitochondria[4]. A most unusual and potentially significant consequence of the data is that since both state III respiration and phosphate esterification decline to similar extents there is little significant change in the P/O ratio. Thus at a Ca^{2+} concentration of 300 nmol per mg protein, the P/O ratio for succinate is still only about half of that observed in the absence of Ca^{2+}.

Since the partial reactions mentioned in the first few paragraphs reflect aspects of the mechanisms of oxidative phosphorylation, we examined the effect of Ca^{2+} on these reactions. Figure 3 shows the influence of Ca^{2+} on the $^{32}P_i$-ATP exchange activity in intact and sonic ascites mitochondria. Since the data in the literature for the rat liver system is inadequate for the present purposes, experiments carried out with mitochondria from this tissue have also been presented. The gradual decline in activity in the ascites mitochondria is again clearly evident and contrasts with the sharp response seen with rat liver mitochondria at about 100 nmol per mg protein. The response to Ca^{2+} of this exchange in intact ascites mitochondria is almost identical to that observed with sonic preparations suggesting that Ca^{2+} does not interact with the intact ascites mitochondria in the same way as it does with the intact rat liver mitochondria.

The response of the ATPase and ADP-ATP exchange activities to Ca^{2+} in intact and sonic preparations is shown in Fig. 4. The pattern of ATPase activity seen here with intact mitochondria most likely reflects that due to accumulation of the Ca^{2+} (see refs 5 and 8) and not that of the 'irreversible' ATPase seen in other mitochondrial preparations[5]. In the present incubation conditions, sonic particles are unable to accumulate Ca^{2+} and this is reflected in the low ATPase activity seen with these preparations. Also in complete constrast with rat liver mitochondria[4], the ADP-ATP exchange activity in intact ascites mitochondria is completely unaffected by all Ca^{2+} levels up to 350 nmol per mg protein.

A comparison of the present body of data (refs 7, 8 and submitted for publication) with that presented previously[4–6] reveals that mitochondria from Ehrlich ascites tumour cells exhibit a response to Ca^{2+} which is distinctly different from that exhibited by all other mammalian mitochondria so far examined. A number of energy-linked reactions intimately linked to the process of oxidative phosphorylation change completely and irreversibly when mitochondria isolated from most normal mammalian tissue are exposed to excessive Ca^{2+} levels. Such changes are either absent or considerably dampened when tightly coupled mitochondria from Ehrlich ascites tumour cells are exposed to similar high levels of Ca^{2+}, which indicates that these mitochondria are atypical in their ability to tolerate extremely high concentrations of Ca^{2+}. Mitochondria from L1210 mouse ascites tumour cells have also been shown to tolerate high levels of Ca^{2+} (ref 12).

Important evidence reflecting this tolerance is shown in Fig. 1 where a complete lack of increase in the resting rate of state IV respiration was observed even after the accumulation of Ca^{2+} concentrations as high as 280 nmol per mg protein. In reactions such as the $^{32}P_i$-ATP exchange where some change did occur, it was usually gradual and in no way reflected the marked change which occurs at Ca^{2+} levels of about 100–150 nmol per mg protein with, for example, rat liver mitochondria. Indeed, in the ascites preparations the response of the exchange to Ca^{2+} was practically identical in intact and sonic preparations.

The molecular events that occur when Ca^{2+} interacts with normal mammalian mitochondria to induce the (irreversible) modifications to the above energy-linked reactions are unclear. All the reactions are membrane-linked and show maximal sensitivity to Ca^{2+} when the mitochondria are most intact as revealed by the tightness of coupling of phosphorylation

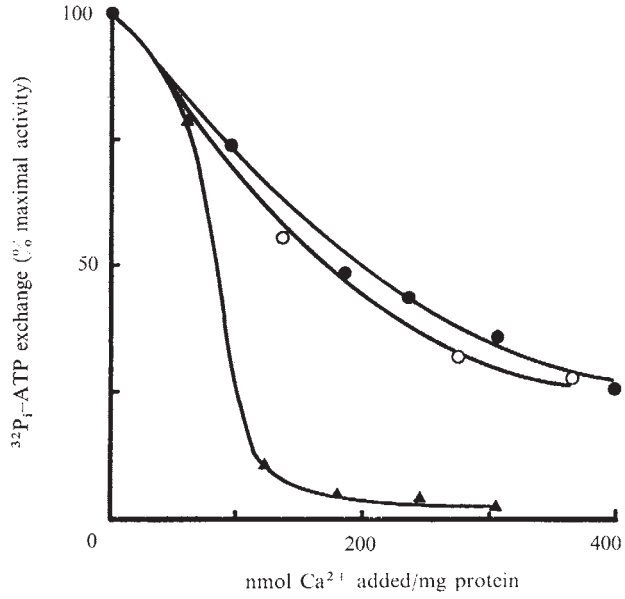


FIG. 3 Influence of Ca^{2+} concentration on the $^{32}P_i$-ATP exchange activity in intact and sonic mitochondria isolated from Ehrlich ascites tumour cells. The experimental conditions were exactly as described by Bygrave and Lehninger[9]. ●, Intact ascites mitochondria (43); ○, sonic ascites mitochondria (11); ▲, intact liver mitochondria (88). Values in parentheses indicate nmol $^{32}P_i$ exchange per min per mg mitochondrial protein in absence of added Ca^{2+}.

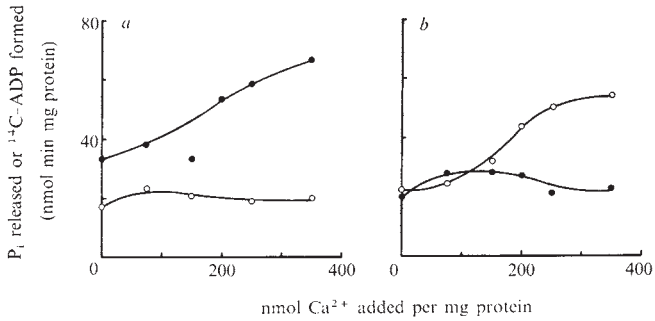


FIG. 4 Influence of Ca^{2+} concentration on ATPase (*a*) and ADP-ATP exchange (*b*) activities in intact and sonic mitochondria from Ehrlich ascites tumour cells. The experimental conditions were exactly as described by Bygrave and Lehninger[9]. ●, Intact mitochondria; ○, sonic mitochondria.

to respiration. Despite the uncertainties of the molecular events involved in uncoupling by Ca^{2+} it is quite clear that the Ca^{2+}-induced mechanism operating in normal mitochondria either does not exist or is grossly modified in mitochondria isolated from Ehrlich ascites tumour cells.

A second significant feature of our data is that the tumour mitochondria are not only tolerant to high levels of Ca^{2+} but also no longer respond to ADP with a respiratory jump. Thus phosphorylating respiration is prevented when these mitochondria are loaded with even small amounts of Ca^{2+}, a feature not seen in normal mitochondria (see ref. 1). This property is being studied in detail in our laboratory and most likely reflects an inhibition of adenine nucleotide translocation by Ca^{2+} in these tumour mitochondria. This is discussed elsewhere (submitted for publication).

Elsewhere we have advocated that Ca^{2+} movements between the cytoplasm and mitochondria form the basis of a means of controlling Ca^{2+}-sensitive cellular metabolic processes located in one or other of these two compartments[13–18]. Thus the unusual interactions of the Ehrlich ascites cell mitochondria with Ca^{2+} described here is of great interest. Although it is difficult to extrapolate information from *in vitro* experiments to *in vivo* events, the intriguing possibility exists that because mitochondria from Ehrlich ascites tumour cells can accumulate and maintain unusually high concentrations of Ca^{2+} without any apparent damage, they provide an environment in the cytoplasm consisting of a high Mg^{2+}:Ca^{2+} ratio. In this way metabolic processes localised in the cytoplasm outside mitochondria such as glycolysis, protein synthesis and phospholipid synthesis, all of which are enhanced by an increase in this ratio[18], proceed much faster than those occurring in other tissues, whose mitochondria are less tolerant to high concentrations of Ca^{2+}. Studies on the relation of these ideas to the growth and metabolic characteristics of various tumours are under way in this laboratory.

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1 Lehninger, A. L., Carafoli, E., and Rossi, C. S., *Advances in Enzymology and Related Areas of Molecular Biology*, **29**, 259 (1967).
2 Carafoli, E., and Lehninger, A. L., *Biochem. J.*, **122**, 681 (1971).
3 Lehninger, A. L., *The Mitochondrion* (Benjamin, New York, 1963).
4 Bygrave, F. L., and Reed, K. C., *FEBS Lett.*, **7**, 339 (1970).
5 Bygrave, F. L., Reed, K. C., and Spencer, T., in *Energy Transduction in Respiration and Photosynthesis* (edit. by Quagliariello, E., Papa, S., and Rossi, C. S., 981 (Adriatica Editrice, Bari, 1971).
6 Saris, N. E. L., *Soc. Sci. fenn.*, **28**, 1 (1963).
7 Thorne, R. F. W., and Bygrave, F. L., *Biochem. biophys. Res. Commun.*, **50**, 294 (1973).
8 Thorne, R. F. W., and Bygrave, F. L., *Cancer Res.*, **33**, 2562 (1973).
9 Bygrave, F. L., and Lehninger, A. L., *J. biol. Chem.*, **241**, 3894 (1966).
10 Morrison, J. F., *Anal. Biochem.*, **24**, 106 (1968).
11 Baginski, E. S., Foa, P. P., and Zak, B., *Clin. chim. Acta.*, **15**, 155 (1967).
12 Reynafarje, B., and Lehninger, A. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1744 (1973).
13 Bygrave, F. L., *Biochem. J.*, **101**, 480 (1966).
14 Bygrave, F. L., *Nature* **214**, 667 (1967).
15 Meli, J., and Bygrave, F. L., *Biochem. J.*, **128**, 415 (1972).
16 Spencer, T., and Bygrave, F. L., *Biochem. J.*, **129**, 355 (1972).
17 Spencer, T., and Bygrave, F. L., *J. Bioenergetics*, **4**, 347 (1973).
18 Bygrave, F. L., *Cellular Calcium and Magnesium Metabolism* in *An Introduction to Bio-Inorganic Chemistry* (edit. by Williams, D. R.) (Butterworths, in the press).

Response of mouse melanoma cells to melanocyte stimulating hormone

WE have recently described a mouse melanoma cell line which responds to melanocyte stimulating hormone (MSH) with dramatic increases in tyrosinase activity and melanin content, as well as changes in cellular morphology and growth characteristics[1–3]. The increase in pigmentation following exposure to MSH is shown in Fig. 1. Cyclic AMP or dibutyryl cyclic AMP may be substituted for MSH in eliciting these responses; consistent with other work suggesting that MSH acts through cyclic AMP in the control of pigmentation in vertebrates[4–7]. In our previous studies we used non-synchronised mouse melanoma cells grown in monolayer culture. Martin *et al.*[8] have shown that synchronised mouse HTC cells respond to corticosteroids with increased tyrosine amino transferase synthesis in the late

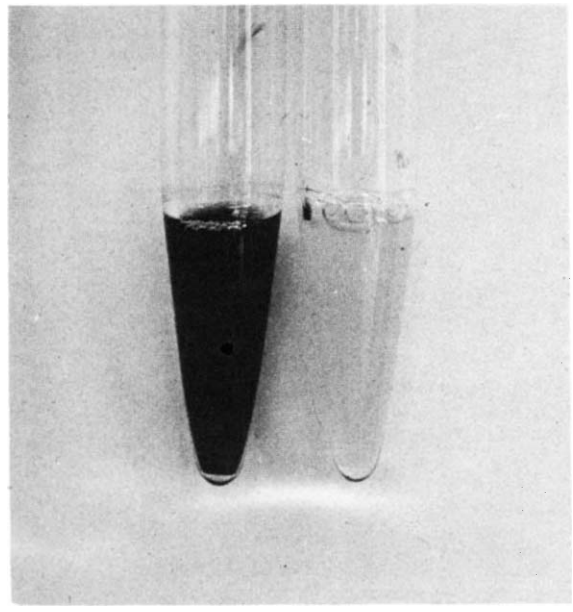


FIG. 1 Effect of MSH on pigment formation by cultured melanoma cells. 10^6 cells were inoculated into each of 4 culture flasks; 10^{-7} M MSH was added to two of the flasks. After 4 d, the cells were collected, pooled, lysed in hypotonic buffer, and photographed. MSH-treated cells are on the left.

TABLE 1 Time periods for the cell cycle of colchicine-synchronised melanoma cells

Hours after release from colchicine	Phase
0–5	Mitosis
5–15	G1
15–25	S
24–45	G2
45–	Mitosis*

* Metaphase cells were counted to determine mitosis. At 45 h, approximately 25% of the cells were in metaphase, at 48 h more than 60% were in metaphase.

G1 and early S periods of the cell cycle; similarly, Buell and Fahey[9] have shown, using a synchronised human lymphoid cell line, that immunoglobulins G and M are expressed in late G1 and S. We wished to investigate the effects of MSH on tyrosinase activity and endogenous cyclic AMP levels in synchronised cells to determine if there is a particular hormone-sensitive phase of the melanoma cell cycle. We report here that melanoma cells responded maximally to MSH with increased tyrosinase activity and cyclic AMP content during the G2 phase.

Cloudman S91 NCTC 3960 (CCL 53) mouse melanoma cells were obtained from the American Type Culture Collection Cell Repository and maintained in monolayer culture as previously described[2]. For a maximal response to MSH, cells were inoculated at 5×10^5 — 10×10^5 cells per 250 ml Falcon tissue culture flask and sub-cultured weekly. Under these conditions, the cells have a doubling time of approximately 48 h. If the cells are inoculated at a higher density or sub-cultured less frequently, they tend to show increased tyrosinase activity and melanin content in the absence of MSH and the response to the hormone is less marked.

Cells were synchronised by exposure to 0.015 μg ml^{-1} colchicine for 36 h, by which time most of the cells had reached metaphase. Metaphase cells were collected by manual shaking of the culture flasks, washed three times in complete medium free of colchicine, inoculated into 100 mm Falcon tissue culture Petri dishes at 10^6 cell per dish in 10 ml medium, and incubated at 37° C in a gassed, humidified incubator. The time periods for the various phases of the cell cycle were determined by the methods of Martin *et al.*[8] Tyrosinase activity was assayed for cells *in situ* as previously described[2].

Cyclic AMP was measured by modifying the method of Brown *et al.*[10]: cyclic AMP binding protein from calf adrenals was further purified on a DEAE-cellulose column so as to be free of phosphodiesterase activity. Theophylline was found to increase the background in the binding assay and was omitted from the reaction mix. Two different procedures were followed to extract cyclic AMP from the cells (see legends to Fig. 4 and Table 2) but the results were essentially the same regardless of which was used.

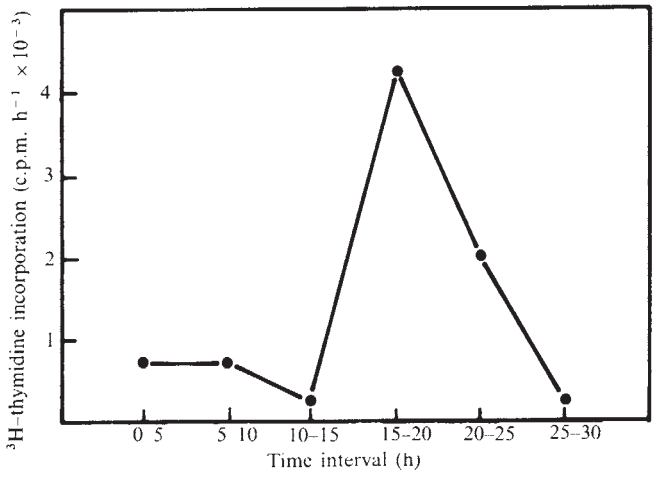


FIG. 2 Incorporation of thymidine ^{3}H-methyl, New England Nuclear 20 Ci mmol^{-1} by synchronised melanoma cells. Cells were incubated with 1 μCi ml^{-1} ^{3}H-thymidine at 5 h intervals. After 5 h in thymidine, the cells were collected, precipitated with cold 5% trichloracetic acid, filtred on to a 0.45 μ Millipore filter, and washed with 30 ml 5% trichloracetic acid containing an excess of non-radioactive thymidine. The filters were dried in air and counted in a liquid scintillation counter. The points are averages of duplicate culture flasks.

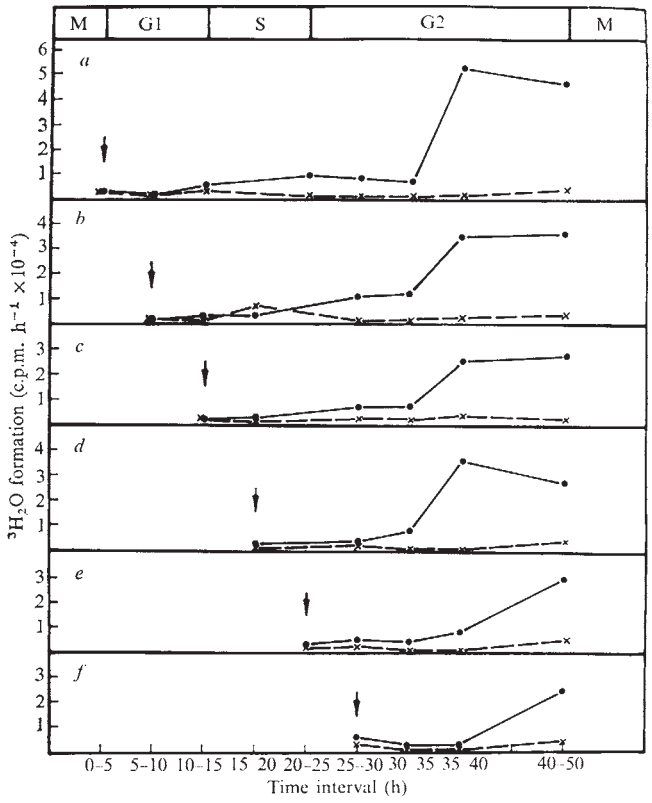


FIG. 3 Tyrosinase activity in synchronised melanoma cells. Cells were incubated with or without 10^{-7} M MSH which was added at the times indicated by arrows. 3, 5-^{3}H-L-tyrosine (New England Nuclear 20 Ci m mol^{-1}) was added at 1 μCi ml^{-1} to control and MSH-treated cultures at the same times that MSH was added. ^3H_2O formation was measured to assay tyrosinase activity. Each point represents the ^3H_2O formed h^{-1} during the various time intervals. Zero time is when the cells were released from colchicine. The points are averages of duplicate culture flasks. ×– – –×, Control; ●—●, MSH treated.

The time periods for the various phases of the cell cycle are shown in Table 1. The incorporation of ^{3}H-thymidine into DNA was used to monitor the S phase (Fig. 2), and metaphase cells were counted to determine mitosis. The periods were the same whether or not MSH was added to the culture medium. MSH (10^{-7}M) was added to the cells at various times after release from the colchicine block in metaphase, and tyrosinase activity and endogenous cyclic AMP levels were followed in control and MSH-treated cells. The experiments were performed at least twice with similar results each time.

Tyrosinase activity was assayed by measuring ^3H_2O released into the medium by cells incubated with 3,5, ^{3}H-L-

TABLE 2 Cyclic AMP levels in synchronised cells following rapid extraction technique*

Time of cyclicAMP Extraction	pmol cyclicAMP per 2 × 10^6 cells			
	Control Experiment	Average	MSH-treated Experiment	Average
t = 3 h	(1) 2.8	3.0	(1) 4.0	5.1
	(2) 3.2		(2) 6.4	
t = 12 h	(1) 2.8	3.8	(1) 3.2	4.0
	(2) 4.8		(2) 4.8	
t = 24 h	(1) 0.8	1.2	(1) 5.4	15.2
	(2) 1.6		(2) 25.0	
t = 30 h	(1) 2.0	1.8	(1) 41.0	43.0
	(2) 1.6		(2) 45.0	
t = 36 h	(1) 2.0		(1) 41.0	
	(2) 3.6	3.3	(2) 65.0	48.0
	(3) 4.2		(3) 37.0	
t = 48 h	(1) 2.4		(1) 25.0	
	(2) 3.0	2.9	(2) 45.0	35.0
	(3) 3.4		(3) 35.0	

* Cells were incubated as described and 10^{-7} M MSH was added 30 min before each sampling. Culture medium was removed by aspiration, the cells were rinsed gently with 2 ml Hanks' balanced salt solution, and ice-cold ethanol–0.2 N HCl was added to the cells with a Pasteur pipette. Removal of medium and addition of ethanol–HCl took less than 10 s. The cells were then placed at −10° C for at least 24 h and thereafter treated as described in legend to Fig. 4. The extracts were diluted in order to measure 0.2 to 5.0 pmol cyclic AMP per assay. The growth medium alone incubated in the presence or absence of MSH or with 100 pmol of added cyclic AMP contributed negligible amounts of cyclic AMP by this extraction procedure.

tyrosine. (Fig. 3*a–f*) Although the additions of MSH were staggered at 5 h intervals from 0–25 h, the hormone-mediated response was always maximal in the G2 phase of the cycle.

Cyclic AMP was measured at each time point following 30 min of exposure to MSH (Fig. 4 and Table 2). Although MSH increased cyclic AMP levels throughout the cell cycle, there was a dramatic rise over control values in the G2 phase of the cycle, 30–40 h after colchicine release. Control values changed very little throughout the cycle. MSH also caused an increase in cyclic AMP released to the culture medium by the cells (Table 3). The sharp increase in cyclic AMP levels in G2 seemed to precede the increase in tyrosinase activity, consistent with previous evidence that cyclic AMP is an intracellular mediator in the MSH response.

Two control experiments not shown here bear on the above results. First, when tyrosinase activity was measured in homogenates of collected cells, the results closely paralleled those obtained by measuring ^3H_2O released to the medium. Thus the lag periods observed in the experiments presented here were not due to a lag in the uptake of ^{3}H-tyrosine by the cells or to a lag in the release of ^3H_2O to the medium. Second, colchicine, added again after the cells had passed through the initial mitosis, did not influence the results, providing additional evidence that the cells responded in the G2 phase.

When non-synchronised cells were treated with MSH, a rise in tyrosinase activity could be detected after a lag period of 6–9 h (ref. 3). The lag period with synchronised cells can, however, be as long as 35 h, depending when in the cell cycle MSH is added (Fig. 3). These results can be explained if it is assumed that in non-synchronised populations, the initial rise in tyrosinase activity takes place in cells that are in the G2 phase of their cycle.

Chen *et al.*[5] have provided evidence that both MSH and cyclic AMP induce the differentiation of melanoblasts into melanocytes in organ cultures of caudal fins from xanthic goldfish. The melanoblasts are inducible shortly preceding mitosis, which may indicate that these cells are also most sensitive to MSH in the G2 phase. This result raises the possibility that vertebrate melanocytes are, in general, responsive to MSH in G2, an hypothesis which awaits further experimentation.

The relationship of the phase of the cell cycle to cellular phenotypic expression is as yet poorly understood. Varga *et al.*[11] have, however, recently provided evidence which explains why the melanoma cells respond to MSH only in the G2 phase. Using ^{125}I-labelled MSH as a tracer, they found that the cells are able to bind the hormone to their plasma membranes only in the G2 phase; there is little or no binding in G1 or S. Apparently MSH-receptors are available only in G2. Thus the MSH-mediated rise in intracellular cyclic AMP concentration is restricted to the G2 phase because this is the only time when the hormone can bind to the cells and stimulate membrane-bound adenyl

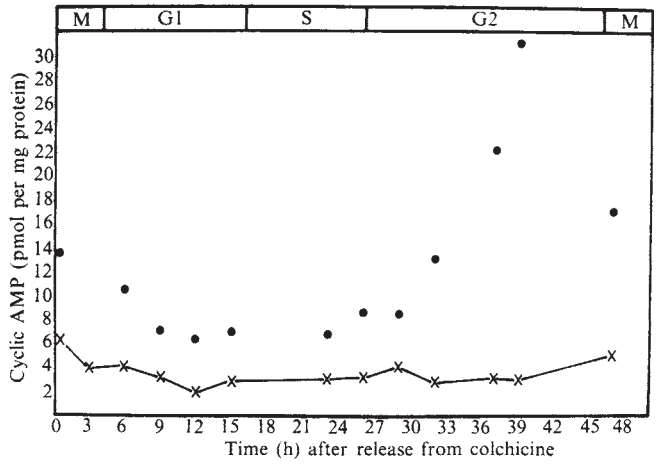


FIG. 4 Cyclic AMP levels in synchronised melanoma cells. Cells were incubated as described and 10^{-7} M MSH was added 30 min before each sampling. The wash procedure took 10 min and all operations were at 4° C. Growth medium was removed, cold Hanks, balanced salt solution was added, the cells were collected with a rubber policeman, washed three times in Hanks' by centrifugation, and cold ethanol containing 0.2 N HCl was added to extract cyclic AMP. After at least 24 h at −10° C the extracts were centrifuged, the supernatants removed, the pellets washed with additional ethanol-HCl, the supernatants pooled, dried *in vacuo*, and cyclic AMP measured by the method of Brown *et al.*[10]. Known amounts of cyclic AMP were added to aliquots of each extract as internal standards. Recovery of the standards was routinely more than 90%. The growth medium alone incubated in the presence or absence of MSH or with 100 p mol of added cyclic AMP contributed negligible amounts of cyclic AMP by this extraction procedure. The pellets were dissolved with 0.1 N NaOH and protein was estimated by the Lowry method. The points are averages of duplicate culture flasks ×--×, control; ● MSH treated.

TABLE 3 Cyclic AMP levels in the culture medium*

Treatment of medium†	pmol cyclic AMP per 10 ml
Without cells and without MSH	Experiment 1 < 0.1
	2 < 0.1
Without cells, MSH added 30 min before sampling	Experiment 1 < 0.1
	2 < 0.1
With cells and without MSH	Experiment 1 < 4
	2 < 4
With cells, MSH added 30 min before sampling	Experiment 1 56
	2 72

* Constituents of the culture medium were listed previously[2]. Cells were released from colchicine and 0.25 ml medium was removed after 42 h incubation at 37° C. A sample of 2 ml ice cold ethanol–0.2 N HCl was added, and the mixture was placed at −10° C. After 24 h the mixtures were centrifuged and thereafter treated as in legend to Fig. 4. The extracts were diluted as described in legend to Table 2.

† All media were incubated for 42 h.

cyclase. Presumably the increased cyclic AMP concentration then results in increased tyrosinase activity.

We do not yet know if the increase in tyrosinase activity in cells exposed to MSH is due to new synthesis of tyrosinase molecules or activation of pre-existing molecules. Localisation of the melanoma cell response to MSH in the G2 phase, however, should be helpful in the identification of the molecular intermediates involved.

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1 Wong, G., and Pawelek, J., *J. cell Biol.*, **55**, 288 (1972).
2 Wong, G., and Pawelek, J., *Nature new Biol.*, **241**, 213 (1973).
3 Pawelek, J., Wong, G., Sansone, M., and Morowitz, J., *Yale J. Biol. Med.*, **46**, 430–443 (1973).
4 Bitensky, M. W., and Demopoulos, H. B., *J. invest. Derm.*, **54**, 83 (1970).
5 Chen, S., Tchen, T. T., and Taylor, J., *Pigment Cell* **1**, 1 (1973).
6 Johnson, G. S., and Pastan, I., *Nature new Biol.*, **237**, 267 (1972).
7 Kreider, J. W., Rosenthal, M., and Lengle, N. *J. natn. Cancer Inst.* **50**, 555 (1973).
8 Martin, D., Tomkins, G. M., and Granner, D., *Proc. natn. Acad. Sci., U.S.A.*, **62**, 248 (1969).
9 Buell, D. N., and Fahey, J. L., *Science*, **164**, 1524 (1969).
10 Brown, B. L., Albano, J. D. M., Ekins, R. P., and Sgherzi, A. M., *Biochem. J.*, **121**, 561 (1971).
11 Varga, J. M., DiPasquale, A., McGuire, J. S., Lerner, A. B., and Pawelek, J., *Proc. natn. Acad. Sci. U.S.A.* (in the press).

Stimulation of tumour cell dissemination by raised temperature (42°C) in rats with transplanted Yoshida tumours

HYPERTHERMIA, that is, temperatures ≥40° C, may have a selective destructive effect on some types of cancer cells[1,2], and sporadic attempts have been made to treat human cancer by raised temperatures[1–3]. Rossi-Fanelli and colleagues have demonstrated a selective and irreversible inhibition of metabolism in a variety of tumour cells at 42° C, whereas normal cells were unaffected[1,4,5]. These workers obtained histological regression of malignant limb tumours in patients treated by regional perfusion with prewarmed blood[1]. More effective and controlled methods for inducing hyperthermia[6], and reports that the action of cytotoxic drugs can be potentiated by hyperthermia[7,8], have further stimulated interest in this approach to the treatment of human malignant tumours.

Little precise information is available on optimal conditions of heating, susceptibility of various tumours, and relative effectiveness of locally applied heat compared with total body heating. Some of these problems have previously been investigated using the VX2 carcinoma in the rabbit[9–11], and we now report observations on the behaviour of the Yoshida tumour in response to elevated temperatures *in vitro* and *in vivo*, with particular reference to the unexpected finding of stimulation of malignant cell dissemination by inadequate heating of implanted tumours at 42° C.

In the present work, the solid form of the transplantable Yoshida tumour[12] in the rat was used. The tumour was maintained by periodic transfer of 0.1 ml (100 mg) tumour homogenate by trocar superficially into the muscle of the hind leg in outbred Wistar rats of both sexes fed an *ad libitum* diet, and weighing 200–250 g. The tumours became palpable after 6 d and increased in volume exponentially until 11 d, when the growth rate decreased in accordance with the Gompertz function for solid tumours. The animals died at 26 ± 3.1 d (260 rats) following inoculation, when the primary tumour volume was approximately 100 ml.

The sensitivity of the Yoshida cells *in vitro* to increased temperature was assessed by conventional Warburg manometry, using 3.0 ml Krebs-Ringer-phosphate buffer, *p*H 7.40, containing 0.013 M sodium succinate with air as the gas phase, and 0.2 ml 10% KOH in the centre well (respiration); for anaerobic glycolysis the suspending buffer was 3.2 ml Krebs-Ringer-bicarbonate-phosphate[4], *p*H 7.40, containing glucose (2 g l^{-1}), with a gas phase of 95% N_2 and 5% CO_2. At temperatures above 41° C, there was depression of both respiration and glycolysis. At 42° C, irreversible damage to the cells occurred after 4 h, as indicated by cessation of O_2 uptake and CO_2 production, failure to respire or glycolyse on transfer to fresh incubation buffer at 38.0° C, and failure to produce tumours when transplanted into rats. On the basis of these results, 42° C was selected as the most suitable temperature to use for therapy of this tumour. Above 42° C, more rapid inhibition of respiration and glycolysis occurred, but the differential heat sensitivity between cancer cells and normal cells is diminished[13], and subject tolerance in animals and in humans dictates that the therapeutic temperature range in heated tumours is 41–42° C[10].

Figure 1 illustrates the temperature profiles obtained when tumour-bearing rats were treated by immersion of the affected limb in a waterbath to maintain an intra-tumour temperature of 42° C for 1 h. It was not possible to achieve this without the animal's central body temperature rising to an average in excess of 41.5° C over the 60 min therapy period. The rats did not tolerate this elevated temperature; of 167 rats so treated, 151 died during heating or within

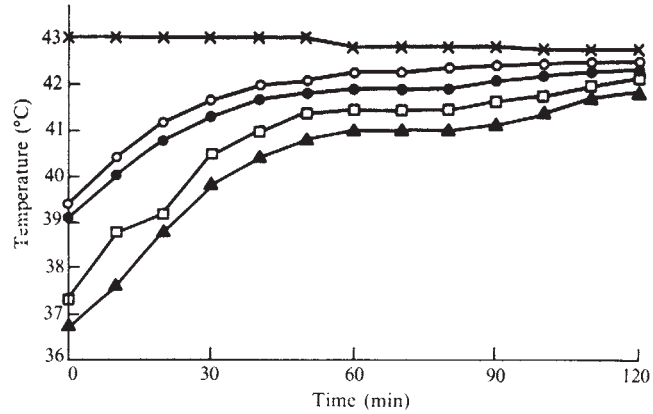


FIG. 1 Tumour and central body (core) temperature profiles for a rat treated by immersion of the tumour-bearing limb in a waterbath at 43°C. The tumours were treated on the eighth day after inoculation into the hind limb when the volume was 10.3 ± 1.3 ml, and there was no necrosis present. The temperature monitoring probes were inserted under Nembutal anaesthesia, and temperatures were measured by multi-channel direct reading electric thermometers (Model 3 GID, Light Laboratories, Brighton). Temperatures were recorded from opposite poles of the tumour (intratumour I and II), from the abdomen and rectum. The rats were heated in groups of four, and during and immediately following therapy each animal was given 1 ml 4% dextrose—0.18% NaCl solution (at 42°C) to replace fluid loss. Temperature: ×, bath; ○, intratumour I; ●, intratumour II; □, intra-abdominal; ▲, rectal.

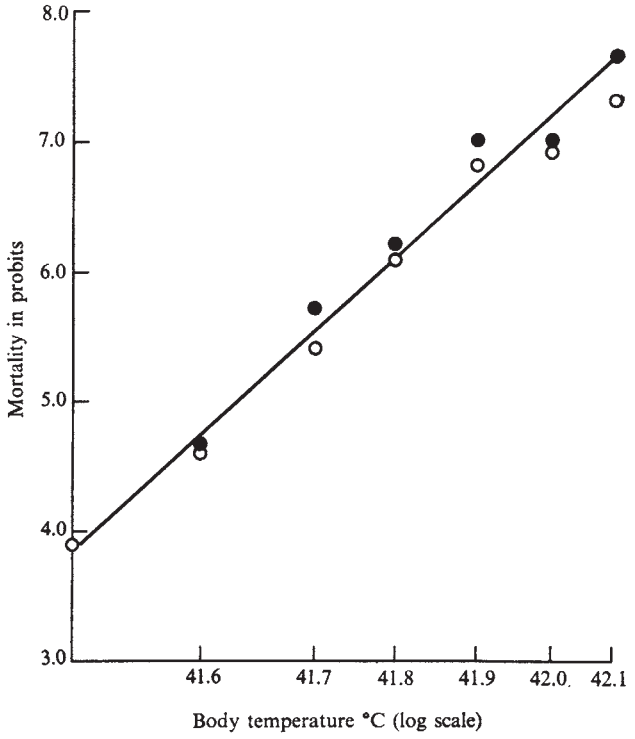


FIG. 2 Probit transformation for % mortality in a series of 200–250 g rats subjected to hyperthermia. The hindquarters of the normal animals were placed in the water-bath, and the temperature in the muscle of the immersed limbs maintained at 42°C for one hour; the tumour-bearing rats were heated as detailed in the legend to Fig. 1. The average body temperature over the heating period was adjusted to the nearest 0.1°C. At each temperature point on the curve for normal rats there were at least eight animals with thirty animals at the 42.0°C point (total number of rats heated = 82), while for the tumour-bearing series there were at least twelve animals at each temperature point, and at 41.9°C and above there were at least thirty animals per point (total number of rats heated = 167). ○, Normal; ●, tumour-bearing rats.

the following 24 hours (survival rate for the heating = 9.6%). One rat survived for three days and nine died at 6–12 d following heating; the remaining six survivors were electively killed for pathological examination at 2–6 d after hyperthermia. As indicated in Fig. 2, the survival rate decreased rapidly when body temperatures in excess of 41.5° C were maintained for 1 h, and there was no difference in susceptibility to heat between normal rats and tumour-bearing animals. A body temperature just over 41.6° C represented the 50% lethal dose of heat, and few rats survived an intra-abdominal temperature of 42.0° C for 60 min.

There was no restraint of tumour growth and no inhibition of respiration after heating. Anaerobic glycolysis was significantly reduced immediately after heating, but returned to control values within 3 d. After hyperthermia, the tumours were oedematous and dark red in colour, and showed little microscopic evidence of cell destruction. These changes have been described previously for inadequately-heated VX2 tumours in the rabbit[11]. In the animals that lived longer than 24 h, the mean survival time was 7.9 ± 2.5 d after hyperthermia, that is the rats died at 15.9 ± 2.5 d after transplantation, which was significantly less than the survival time in untreated animals ($P < 0.001$). Non-tumour-bearing rats that survived the hyperthermia had a normal life span.

Following implantation into the leg, the Yoshida tumour infiltrated muscle and other tissues, bone and the overlying skin. Later there was direct spread into the muscles of the pelvis and posterior abdominal wall. From the nineteenth day after implantation, there was tumour in the retroperitoneal tissues in 38.7% of the animals (Fig. 3). In some rats the tumour had formed a large mass in the posterior abdominal wall surrounding and infiltrating both ureters. The perinephric and (or) peri-adrenal fat was infiltrated, and in advanced cases tumour had spread from the mesentery into the wall of the small intestine.

There was spread of tumour to the iliac lymph nodes by the ninth day, and later to the intra-abdominal para-aortic nodes. Tumour was observed in the upper para-aortic nodes at the level of the coeliac axis from the fourteenth day onwards in 32.4% of animals (Fig. 3). In animals with advanced tumours, the deep cervical lymph nodes adjacent to the thymus were involved, and in some, tumour had spread into surrounding fat and into the thymus.

Distant blood-borne metastases were uncommon in that pulmonary tumour emboli (Fig. 4) were observed in only two of ninety-one rats at 22 and 27 d after implantation, respectively. Involvement of the heart was commoner, and tumour was present in the myocardium in seventeen of sixty-nine rats (24.6%) with 13 to 32-d-old tumours (Fig. 3).

The liver and brain were not involved in any of the control animals.

The sixteen animals which survived heating at 42° C for 24 h or longer showed a more rapid dissemination of tumour than did unheated or sham-heated controls. This applied to direct, lymphatic, and blood-borne spread of the tumour (Fig. 3). Involvement of the retroperitoneal tissues occurred earlier, and with a significantly increased incidence, in the heated compared with the control series of animals over the same period (10–20 d following tumour implantation). Similarly, upper para-aortic lymph node involvement was observed earlier in the heated animlas. Myocardial involvement was not premature, but occurred in a greater proportion of heated rats in comparison with the controls. Pulmonary involvement by tumour was observed in three of the sixteen heated rats, a much higher incidence than that in the controls. In two animals at 14 and 15 d following tumour implantation, there were multiple pulmonary emboli, and in the third, the lung parenchyma was extensively involved from the hilar aspect by mediastinal tumour which also involved the heart.

A further distinction between the control and test rats was the involvement of the portal tracts of the liver in two of the heated animals at 14 and 15 d, respectively, after tumour implantation (Fig. 5).

To exclude the possibility that manipulation of the animals and insertion of thermosensor needles into the tumour during heating might adversely affect spread of the tumour, a group of eighteen rats with 8-d tumours was heated in a bath at body temperature (38° C) for 2 h with needle probes inserted into the tumour. These sham-heated rats survived the procedure without loss and seven were killed at the fifteenth day, ten at the twenty-second day, and the remaining animal at the twenty-ninth day following tumour implantation. *Post mortem* examination of these animals showed a similar pattern of tumour spread in relation to the duration of the tumour implant as that observed in the control series (Fig. 3).

With various tumours transplanted subcutaneously in rats[13,14] and mice[15,16], other workers have shown that the destructive effects of hyperthermia on susceptible tumours *in vivo* depend upon both the degree of heat and its duration. The response of the tumour can be expressed as an exponential type of curve in which approximately the same biological effect is obtained by halving the exposure time for each degree that the temperature increases above 42° C. To obtain regression in a variety of small tumours (maximum volume 2 ml) transplanted subcutaneously in rats, an exposure time of 60–90 min at 45° C is required[13,14].

The thermoregulatory mechanisms in rodents are less well-developed than those of man[2], and the animals are more sensitive to hyperpyrexia. The size of the Yoshida tumour (about 10 ml) in relation to the rat's body size implies that a considerable volume of blood (total blood volume in a 200 g rat = about 10 ml or 4.5% body weight[17]) is contained within the immersed tumour-bearing limb at any given instant. This precludes the maintenance of a temperature differential between tumour and normal body organs compatible with survival. A further difficulty encountered

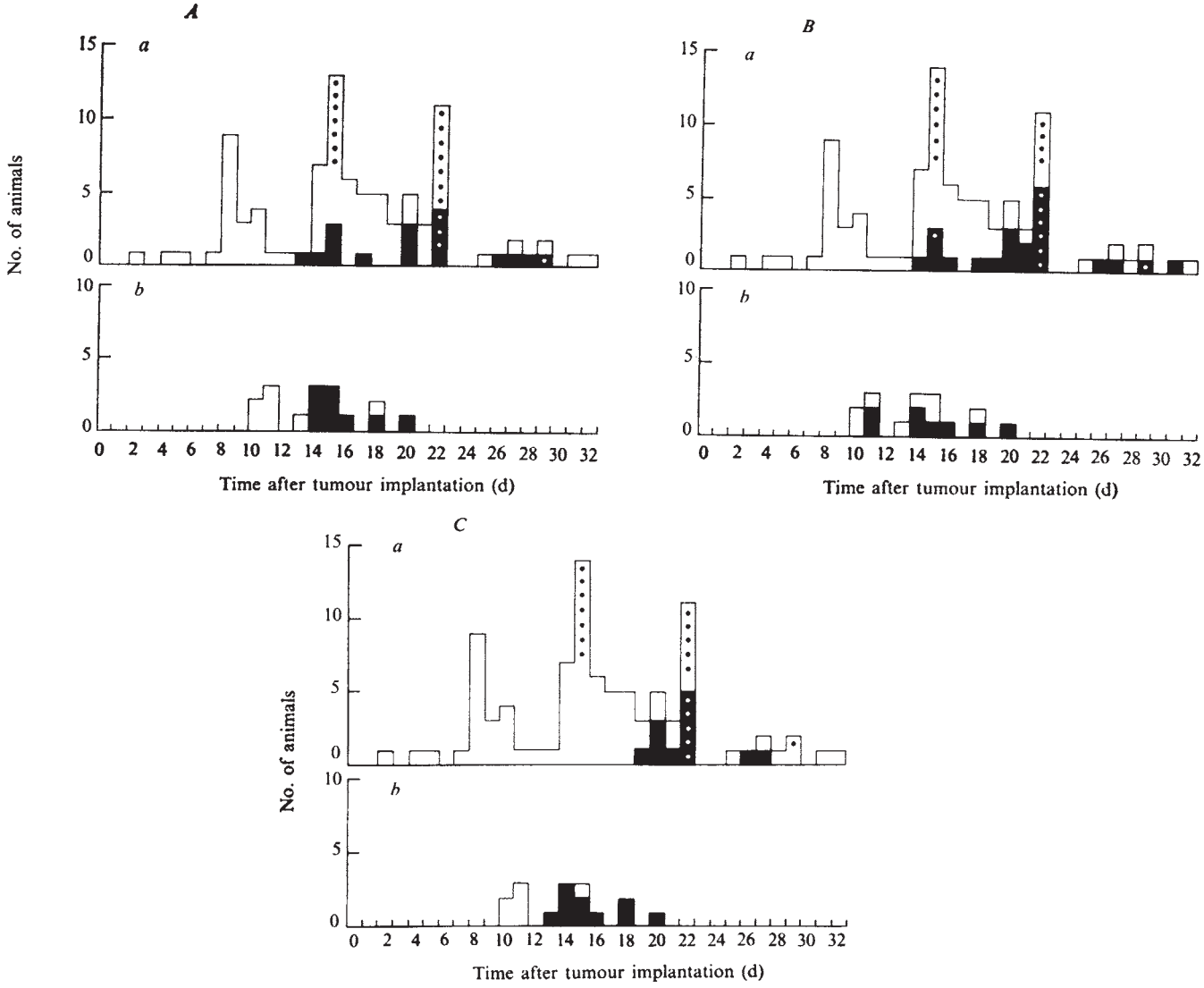


FIG 3 Incidence of tissue involvement by tumour. Examples illustrate spread by the direct, lymphatic and vascular routes. Animals were examined at post-mortem and in each of the 107 rats depicted in these frequency diagrams, the brain, abdominal and thoracic organs were examined histologically. The control and sham-heated animals were killed at intervals after implantation of the tumour to follow the time sequence of tumour spread by the various routes. All heated rats died by the twentieth day after implantation, with the exception of six animals that were electively killed to ascertain the pattern and extent of tumour spread prior to the fifteenth day. In animals that died during or within 24 h of heating, the post-mortem and histological findings did not differ from those in equivalent control rats.

In the case of the heart (*A*), control (*a*) and heated (*b*) animals showed myocardial involvement by tumour at the same time interval after implantation of the tumour (days 13 to 14). The incidence of heart involvement over the 10–20 d period in the heated animals was significantly higher than in the controls ($P < 0.01$). Note also that at the fifteenth day, none of the sham-heated animals showed myocardial involvement, and even at the twenty-second and twenty-ninth days, only four of eleven such animals had myocardial metastases. The incidence of myocardial involvement in the heated animals from the fourteenth to the twentieth days was nine out of ten. This suggests the increased incidence of myocardial involvement in the heated animals was not the result of mechanical factors associated with the heating procedure, such as the insertion of the needle thermistors into the tumour.

The para-aortic lymph nodes (*B*) at the level of the coeliac artery, and alongside the kidneys, were involved earlier in the heated animals (*b*) (eleventh day onwards) than in the controls (*a*) (fourteenth day onwards). The overall incidence over the 10–20 d period was thus higher in the heated group (50%) than in the controls (14.2%), and this difference is significant ($P < 0.02$). Insertion of the thermosensors again was without effect, and only one of seven sham-heated rats at 15 d (14.3%) had upper para-aortic lymph node involvement. In unheated and sham-heated animals with older tumours, the incidence of lymph node metastases was similar to that in heated animals at an earlier period after implantation and occurred in ten of twenty rats from the days 22–32 (50%).

The infiltration of retroperitoneal tissues (*C*) followed a similar pattern, with earlier involvement in the heated rats (*b*) at the thirteenth day compared with the nineteenth day in the controls (*a*), and with a significantly higher incidence in the 10–20 d period in the heated animals (62.5% compared with 7.7%, $P < 0.001$). In the heated rats showing this involvement, a large tumour mass was also present on the posterior abdominal wall. The tumour usually extended up to the lower pole of the kidneys, and often invaded the ureters. Again the sham-heated animals behaved as the other controls.

□, No tumour involvement; ■, tumour present; ●, sham-heated controls.

by previous workers has been inhomogeneous heating of tumours[13,14]. In our work, a temperature difference of up to 0.5° C was detected by electrodes simultaneously inserted into the four quadrants of the same tumour during heating. The superficial temperature of the tumour was usually higher than the deep temperature. Two needles inserted into opposite poles to a depth of 1 cm gave a reliable guide to the temperature in the centre of the neoplasm. The depression of Yoshida tumour metabolism at 42° C *in vivo* was reversible, and together with the absence of histological damage and subsequent unrestrained increase in tumour volume, indicates that the hyperthermia was inadequate for tumour destruction.

The advanced stage of the disease in the rats that survived hyperthermia indicates that the heating accelerated dissemination of this tumour. The precise mechanisms involved are undetermined. Metastasis is a complex phenomenon comprising a sequence of at least five processes[18,19]. The hyperdynamic state of the circulation at elevated temperature, and the local active hyperaemia, may predispose to increased 'showering' of tumour cells into the blood from the primary mass[20]. It is now recognised, however, that there is no definite correlation between the presence of cancer cells in the blood *per se* and the development of metastases[18,19]. Local hyperthermia may have changed the mobility of the cells of the primary tumour. Mondovi *et al.*[21] recently described an increased antigenicity in Ehrlich ascites cells heated at 42.5° C *in vitro* for 1–3 h, and postulated that short exposure to raised temperatures caused some modification of the cell surface. The rise of central body temperature to more than 41.5° C may have altered the metabolic or biological properties of the tissues concerned

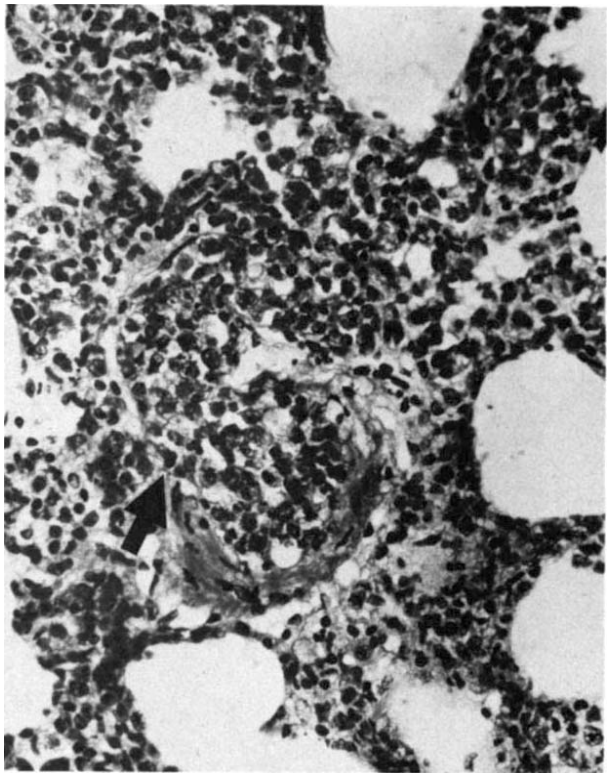


FIG. 4 Pulmonary tumour embolism in control rat killed 22 d after implantation of tumour into the leg. Note tumour cells occluding the lumen of a pulmonary vessel and spreading into the alveolar wall capillaries (arrow). This phenomenon was observed in only two rats in the large control series (ninety-one animals) at 22 and 27 d after tumour transplantation, respectively, but occurred in two of the sixteen heated rats dying at 14 and 15 d after implantation, respectively. Haematoxylin and eosin ×200

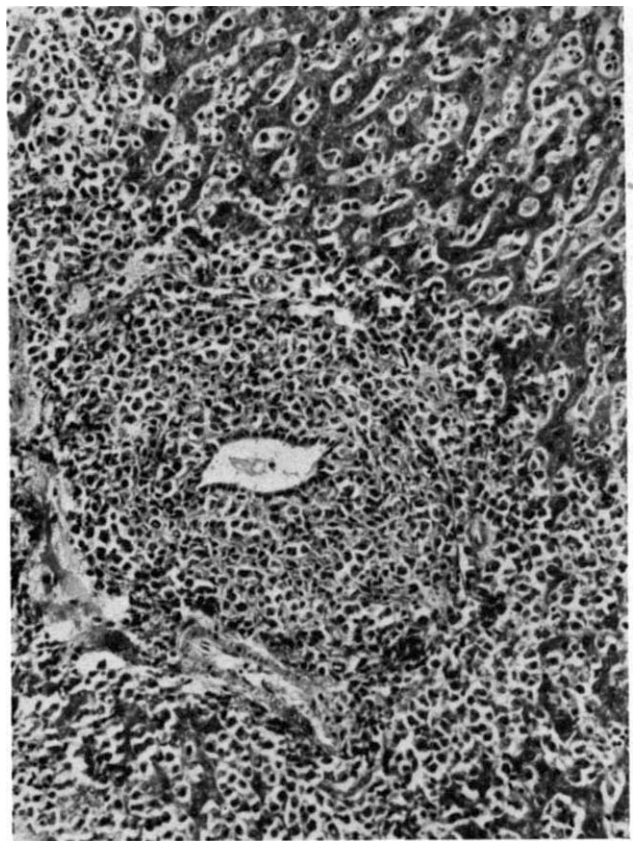


FIG. 5 Tumour infiltration of portal tract and sinusoids in liver of rat heated at 42°C for 1 h, 8 d after implantation of Yoshida tumour into the leg. Death occurred 6 d later. There was also metastatic tumour in the heart, and widespread tumour throughout the retroperitoneal tissues involving the bowel wall and renal parenchyma. Haematoxylin and eosin ×120

in metastatic distribution of the cancer—the so-called 'soil' originally discussed by Paget[22]. Histological examination is a relatively crude method of detecting the presence of metastases[23,24]; it is therefore possible that the present results reflect stimulation of premature activity in Yoshida cells already present in metastatic sites at the time of heating. The appearance of metastases in the liver of two of the sixteen heated animals would seem to argue against this interpretation. A possible depression in the immune defence mechanisms of the host at increased body temperature[10] leading to more rapid spread of the disease must also be borne in mind.

In our work, the Yoshida tumour behaved as a highly-malignant rapidly growing sarcoma, with a capacity for metastasis not commonly found in transplantable animal tumours, but more usually associated with malignant neoplasia in man[19]. Although the various factors controlling the selective localisation and development of secondary tumours have not yet been clearly defined, the pathogenesis of a given neoplasm is influenced by a balance operating between the growing tumour and the reaction of the host[23]. Depending on the adequacy of heat application to the primary tumour, this balance can be altered to the benefit or detriment of the host. It has been claimed that hyperthermia is most effective against rapidly growing tumours of mesenchymal origin[1,2,8,15]. In the light of our present experiences, however, the possible hazards of inadequate heating of malignant tumours in animals and in man must be considered.

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1 Cavaliere, R., Ciocatto, E. C., Giovanella, B. C., Heidelberger, C., Johnson, R. O., Margottini, M., Mondovi, B., Moricca, G., and Rossi-Fanelli, A., *Cancer,* **20,** 1351 (1967).
2 Vermel, E. M., and Kuznetsova, L. B., *Problems of Oncology,* **16,** 96 (1970).
3 Nauts, H. C., Fowler, G. A., and Bogatko, F. H., *Acta med. Scand. Suppl.,* **276,** 1 (1953).
4 Mondovi, B., Strom, R., Rotilio, G., Agro, A. F., Cavaliere, R., and Rossi-Fanelli, A., *Eur. J. Cancer,* **5,** 129 (1969).
5 Mondovi, B., Agro, A. F., Rotilio, G., Strom, R., Moricca, G., and Rossi-Fanelli, A., *Eur. J. Cancer,* **5,** 137 (1969).
6 Henderson, M. A., and Pettigrew, R. T., *Lancet,* **i,** 1275 (1971).
7 Giovanella, B. C., Lohmann, W. A., and Heidelberger, C., *Cancer Res.,* **30,** 1623 (1970).
8 Stehlin, J. S., *Surg. Gynecol. Obstet.,* **129,** 305 (1969).
9 Muckle, D. S., and Dickson, J. A., *Br. J. Cancer,* **25,** 771 (1971).
10 Dickson, J. A., and Muckle, D. S., *Cancer Res.,* **32,** 1916 (1972).
11 Muckle, D. S., and Dickson, J. A., *Br. J. Cancer,* **27,** 307 (1973).
12 Stewart, H. L., Snell, K. C., Dunham, L. J., Schlyen, S. M., *Transplantable and Transmissible Tumors of Animals* (Armed Forces Inst. Path., Washington, D.C., 1959).
13 Westermark, N., *Scand. Arch. Physiol.,* **52,** 257 (1927).
14 Johnson, H. J., *Am. J. Cancer,* **38,** 533 (1940).
15 Crile, G., *Cancer Res.,* **23,** 372 (1963).
16 Overgaard, K., and Overgaard, J., *Eur. J. Cancer,* **8,** 65 (1972).
17 Rowett, H. C. Q., *The Rat as a Small Animal,* 40 (John Murray, 1960).
18 Weiss, L., *The Cell Periphery Metastasis and Other Contact Phenomena* (North Holland Publishing Co., Amsterdam, 1967).
19 Foulds, L., *Neoplastic Development,* **1** (Academic Press, London and New York, 1969).
20 Griffiths, J. D., and Salsbury, A. J., *Br. J. Cancer,* **17,** 546 (1963).
21 Mondovi, B., Santoro, A. S., Strom, R., Faiola, R., and Rossi-Fanelli, A., *Cancer,* **30,** 885 (1972).
22 Paget S., *Lancet,* **i,** 571 (1889).
23 Romsdahl, M. D., Chu, E. W., Hume, R., and Smith, R. R., *Cancer,* **14,** 883 (1961).
24 Greene, H. S. N., and Harvey, E. K., *Cancer Res.,* **24,** 799 (1964).

Recovery of immune system after cigarette smoking

Recent evidence has shown that the immune response in human cigarette smokers[1] and in mice chronically exposed to fresh cigarette smoke[2–4] is impaired. It has been proposed that depressed immunity in smokers may therefore be involved in the increased prevalence of respiratory infection and neoplasia associated with this habit[5].

In this laboratory, mice exposed to cigarette smoke show a depression in the development of antibody-producing cells in the spleen, the lungs and their draining lymph nodes, after the introduction of sheep erythrocytes (SRBC) into the respiratory system[2,3]. Both primary and secondary responses are depressed. This is accompanied by a loss in the phytohaemagglutinin (PHA) responsiveness of the lymphocytes, particularly in the lymph nodes of the respiratory tract[4]. Moreover, the smoke-exposed mice developed an increased number of macrophages in the lungs[6] similar to that observed in human smokers[7]. Although an infiltration of lymphocytes into the lungs also occurs in mice soon after the start of exposure to smoke, it is only transient[6].

The lungs and livers of mice exposed to the smoke also show elevated activity of aryl hydrocarbon hydroxalase[8], an enzyme which also exhibits high activity in the tissues of human smokers[9]. In other experiments[10], alveolar macrophages lavaged from mice immediately after exposure to smoke exhibited depressed viability, and surviving cells showed altered metabolic activity, indicating penetration of active components of cigarette smoke deep into the lungs. Furthermore, histological sections of lungs from these exposed animals show tar-laden macrophages in the alveoli.

The impairment in the humoral immune response appears to be more complex than just a depression in the amount of immunogen absorbed through the respiratory tract. The antibody-producing cell response at a distant site, the spleen, is less sensitive to the effects of cigarette smoke than sites associated with the respiratory system[2]. A defect in lymphocyte function, as indicated by their impaired responsiveness to PHA (ref. 3), provides a possible mechanism.

While it is important to determine the severity and the mechanisms by which immunosuppression is induced by cigarette smoke exposure, it is equally important to investigate whether or not these effects are reversible on termination of cigarette smoking. We present evidence here which indicates recovery of humoral immunity on termination of cigarette smoke inhalation.

Groups of mice were exposed to fresh cigarette smoke in a Hamburg II small animal smoking machine (Heinr. Borgwaldt, West Germany) on week days. Smoking conditions were as previously described[2]. To measure the humoral immune response, mice were inoculated intratracheally with 10^8 SRBC, and after 7 d the direct and indirect plaque-forming cells (PFC) in the spleen, the lungs and a pool of the mediastinal and cervical lymph nodes were determined by the Cunningham-Szenburg modification of the Jerne plaque technique as previously described[2]. The

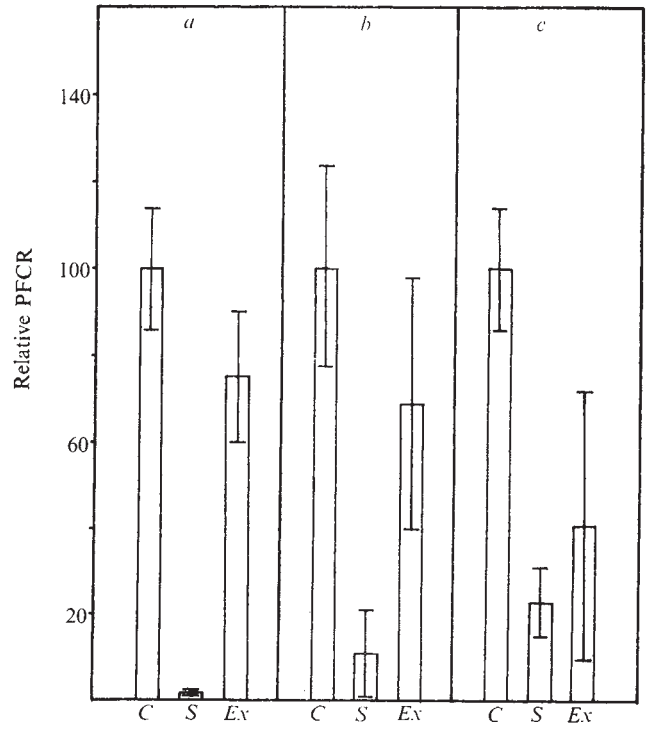


Fig. 1 Recovery of the immune responses of mice to sheep erythrocytes following the cessation of smoking. The plaque-forming cell response (PFCR) before (S), and after, *Ex*, cessation of cigarette smoke exposure is shown (mean ± s.e) as a % of age-matched controls *C*. *a*, Lung; *b*, lymph node; *c*, spleen.

number of PFC per 10^6 leukocytes was calculated. About 10^8, 10^7 and 10^6 leukocytes (predominantly lymphocytes) per cell preparation were obtained from the spleen, lymph nodes and lung respectively. No difference between the number of leukocytes in the cell preparations from normal mice and mice exposed to smoke could be detected. The assay time used coincides with the peak primary response of normal mice. Groups of five to nine mice were used in all experiments and the significance of differences between groups was determined by Student's t test.

The direct PFC response in mice exposed to fresh cigarette smoke for 42 weeks (S), together with the response of mice exposed to fresh cigarette smoke for 42 weeks followed by a further 16 weeks during which time they were not exposed to smoke (Ex), are shown as % of age-matched controls (C) in Fig. 1. The control mice had an average of 21 PFC per 10^6 leukocytes in the lung, 80 PFC per 10^6 leukocytes in the lymph nodes and 10 PFC per 10^6 leukocytes in the spleen.

Control mice used here were not sham-smoked, as observations[4,11] indicate that handling stress does not contribute significantly to the effects observed in the exposed mice.

The primary immune response in all organs was markedly depressed after 42 weeks smoke exposure compared with control animals (lungs, $P < 0.05$; lymph nodes, $P < 0.01$; spleen, $P < 0.05$). Sixteen weeks after the cessation of smoking the mice exhibited significantly increased direct PFC responses in the lungs ($P < 0.001$) and the lymph nodes draining the respiratory system ($P < 0.05$) when compared to animals at the end of the 42 week exposure period. Although the average response in the spleen indicated recovery the results observed were extremely variable, with several of the animals still exhibiting low responses. The indirect PFC responses also showed a depression after 42 weeks' smoke exposure in all organs and a recovery 16 weeks after exposure to smoke ceased. The numbers of indirect PFC in the primary responses, however, were low.

The immune system in the respiratory tract exerts a protective function against infections[12,13] and tumour development[14–16]. These diseases show increased prevalence rates in smoking mice[11,17] and in human smokers[18], but human epidemiological studies indicate that cessation of smoking progressively leads to a lowering of the risk of their development[18,19].

If the immune system of man reacts to cigarette smoke in a similar way to that of the mouse, as some data imply[1], the phenomenon of 'recovery' in the ex-smoker[18,19] may be attributable, in part, to a similar restoration of immune function as that demonstrated here.

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1 Finklea, J., Hasselblad, V., Riggan, W., Nelson, W., Hammer, D., and Newill, V., *Am. Rev. resp. Dis.*, **104**, 368 (1971).
2 Thomas, W., Holt, P. G., and Keast, D., *Nature*, **243**, 240 (1973).
3 Thomas, W., Holt, P. G., and Keast, D., *Arch. envir. Hlth*, **27**, 6 (1973).
4 Thomas, W., Holt, P. G., and Keast, D., *Int. Arch. Allergy appl. Immun.* (in the press).
5 Holt, P. G., Thomas, W., and Keast, D., *Lancet*, **i**, 1316 (1973).
6 Holt, P. G., and Keast, D., *Life Sci.*, **12**, 377 (1973).
7 Harris, J. O., Swenson, E. W., and Johnson, J. E., *J. clin. Invest.*, **49**, 2086 (1970).
8 Holt, P. G., and Keast, D., *Experientia*, **29**, 1004 (1973).
9 Welch, R. M., Harrison, Y. F., Gommi, B. W., Poppers, R. J., Finster, M., and Conney, A. H., *Clin. Pharmac. Ther.*, **10**, 100 (1969).
10 Holt, P. G., and Keast, D., *Arch. envir. Hlth.*, **26**, 300 (1973).
11 Leuchtenberger, C., and Leuchtenberger, R., *Morphology of Experimental Respiratory Carcinogenesis, A.E.C. Symposium Series*, **21**, 329 (1970).
12 Fazekas de St. Groth, S., and Donnelly, M., *Aust. J. exp. Biol. med. Sci.*, **28**, 45 (1950).
13 Smith, C., Purcell, R., Bellanti, J., and Chanock, R., *New Eng. J. Med.*, 275, 1145 (1966).
14 Burnet, F., *Immunological Surveillance* (Pergamon Press, Oxford, 1970).
15 Keast, D., *Lancet*, **ii**, 710 (1970).
16 Richters, A., Sherwin, R. P., and Richters, V., *Cancer Res.*, **31**, 214 (1971).
17 Green, G., *Am. Rev. resp. Dis.*, **102**, 691 (1970).
18 *Smoking and Health Now* (Royal College of Physicians, 1971).
19 Wynder, E., *Cancer N.Y.*, **30**, 1332 (1970).

Associative learning of *Drosophila melanogaster*

Through the work of Benzer[1,2], attention has been focussed on behavioural studies of *Drosophila melanogaster* and the possibilities of a genetic approach to the investigation of nervous systems. For example, experiments on phototaxis[1] and optomotor response[3,4] have made possible the selection of behavioural mutants in which electrophysiological[5,6] and neuro-anatomical[7] correlates could be established. With the analysis of the mechanism of information storage and(or)

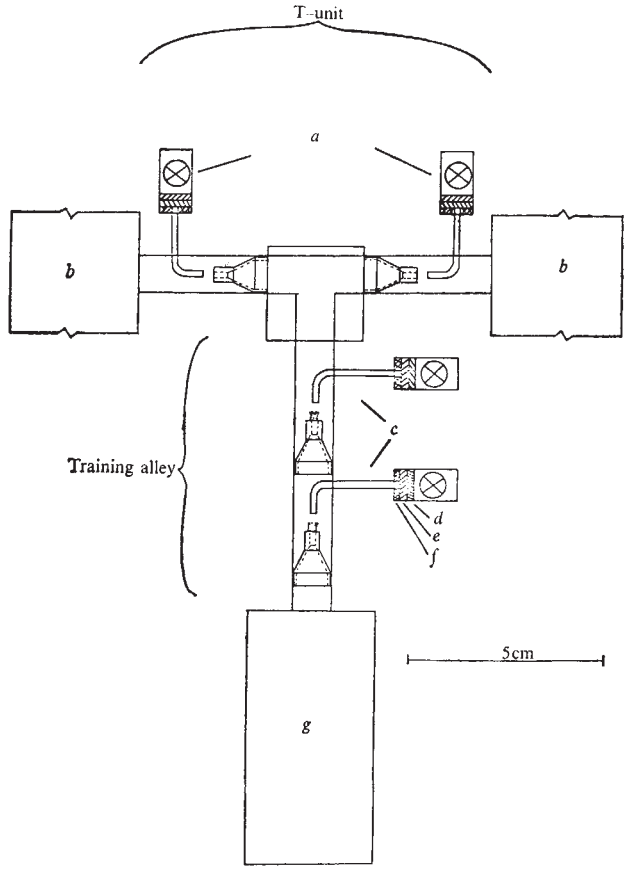


Fig. 1 Schematic drawing of the test apparatus. *a*, lamp; *b*, collecting container; *c*, light conductor; *d*, heat absorption filter; *e*, plexiglass filter; *f*, PVC (grey); *g*, start.

TABLE 1 Results of electroshock experiments in *drosophila melanogaster*

Experiment No.	Electroshock at funnel No.	Number of 'blue' choices	Number of 'yellow' choices	% 'blue' choices	Δ
I	1 (yellow)	95	103	48.0	+ 9.2
	–	69	109	38.7	
	2 (blue)	50	91	35.5	− 6.9
	–	61	83	42.4	
II	2 (yellow)	131	170	43.5	+13.8
	–	84	199	29.7	
	1 (blue)	56	98	36.4	− 9.5
	–	45	53	45.9	
III	2	94	103	47.7	+ 2.3
	–	89	107	45.4	
IV	1	84	88	48.8	− 0.7
	–	101	103	49.5	

The lamp voltage used in experiments I and II, and for the lamps in the T-unit in experiment III, was 4.0 V.

learning as our ultimate goal, we have attempted to demonstrate associative learning with respect to optical stimuli in *Drosophila*. Here we report our first successful experiments.

Attempts to demonstrate spatial discrimination learning of *Drosophila* were reported by Murphey[8,9] but later contradicted by Yeatman and Hirsch[10]. Recently Quinn, Harris and Benzer[11] have found conditioned behaviour in *Drosophila melanogaster* both with olfactory and visual discrimination. Götz (personal communication) has trained individual *Drosophila* with respect to light–dark stimuli. *Drosophila* strains wild type (Berlin) and *ebony* were kept on standard media at 22° C with a 12 h light: 12 h dark cycle. The flies used in the experiments were aged between 1 and 10 d after eclosion (No separation of male and female flies has been undertaken as yet). Because ebony have a higher activity in the maze used, experiments were performed with strain *ebony* unless otherwise stated.

The test apparatus (Fig. 1) is related in design to the T mazes used by Hadler[12]. It rests horizontally on a table and is constructed entirely of plexiglas. The flies are placed in a starting container and can move freely into the 'training alley'. This consists of a tube 13 mm in diameter with two narrow funnels placed 3.5 and 7 cm respectively from the start. The funnels are constructed (Fig. 2) to provide a one-way passage for the flies. In front of each funnel is a plexiglas light conductor, connected to an Osram 2403 (6 V, 1 W) lamp equipped with plexiglas coloured filters (Röhm and Haas No. 300 and 627). Except for the front faces, the light conductors are covered with black tubing. The flies can thus be exposed to a blue or a yellow light, most intensely at the exit of the funnels. After having passed through the training alley the animals reach a 'T-unit' where they can choose to proceed through a yellow or through a blue illuminated funnel constructed and illuminated exactly as in the training alley. After having passed through these funnels the flies are collected in two separate containers and counted conventionally.

Training is carried out with electroshocks. For this purpose the funnels in the training alley are equipped with tiny copper wires (Fig. 2) connected to the output of a pulse generator. The pulses have a duration of 1 ms with 80 V peak voltage at 80 ms intervals. If a fly touches two wires of opposite polarity, it experiences electrical shocks with currents of about 10 μA. The animals clearly try to escape these electroshocks. The construction of the shocking device is the critical part of the apparatus. As recorded with a microammeter only about 30% of the animals, especially the smaller ones, can pass the funnel without being shocked.

In the basic experiment, the flies were exposed to an electroshock at only one of the funnels (either blue or yellow illumination) and the % of animals that passed the 'blue funnel' in the T-unit, relative to the total number that completed the run was determined. This was compared to the % measured without application of electroshocks, that is, the spontaneous distribution measured in a separate experiment in identical optical and geometrical conditions but with a new population of flies. To ensure true difference measurements, this control was always carried out immediately before the test run. Furthermore the apparatus was taken apart, washed with detergent and carefully dried between two different experiments, but not between the control and test runs. In this way slight changes in the apparatus after reassembly, which are reflected in the spontaneous tendency (see Table 1), cancel out in the difference betwen the two runs.

The experiments were carried out at room temperature. Wet filter papers placed in the counting containers helped to maintain an appropriate humidity for the flies and possibly served as attractant as well. Approximately 300 flies were placed in the starting container for each run. At the end of the run, after about 5 h, more than 50% had passed the T-maze. Except for the yellow and blue lamps, the experiments were carried out in the dark. If observation was necessary, overhead red safety lights (Philips TL 40W/15) were used.

For each run the number of animals having chosen the

TABLE 2 Statistical significance of results.

Strain	Electroshock at	Δ	*t*-Test	χ^2-Test	Interpretation
Wild type	'yellow'	+10.7 ± 3.4	$P < 0.01$	$P \ll 0.001$	Avoidance of 'yellow'
	'blue'	−17.6 ± 5.2	$P < 0.01$	$P \ll 0.001$	Avoidance of 'blue'
ebony	'yellow'	+ 9.1 ± 2.9	$P \ll 0.01$	$P \ll 0.001$	Avoidance of 'yellow'
	'blue'	−12.1 ± 2.9	$P < 0.01$	$P \ll 0.001$	Avoidance of 'blue'
ebony	Funnel No. 1 (no lights in training alley)	+ 1.1 ± 4.4	$P > 0.6$	$P > 0.5$	——

The first four experiments listed used the same arrangement of lights. The first funnel was illuminated with yellow light, the second with blue light. The lamp voltage for experiments with wild type is 3.2 V. Ebony is a phototactic mutant[4] a lamp voltage of 4.0 V was used in experiments with ebony. The last experiment listed used no lights in the training alley, but blue and yellow illumination of the funnels in the T-unit.

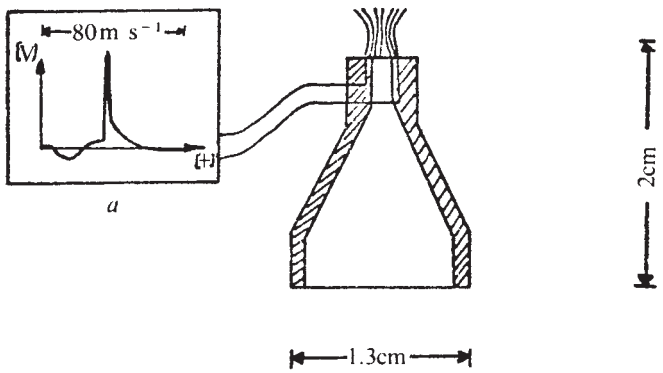


FIG. 2 Schematic drawing of the electroshock device. *a*, Pulse generator.

blue or yellow illuminated arm of the T-unit were counted and the % of 'blue' choices determined. As we have pointed out above, the experiments were performed as difference measurements between a population having experienced electroshocks associated with a particular optical stimulus and a population which had received no electroshocks. A conditioning index can thus be defined as

$$\Delta = (\text{choices})_{\text{shocked}} - (\%\ \text{'blue' choices})_{\text{control}}.$$

This difference is zero, if the animals do not learn. Avoidance of 'yellow' after electroshocks results in positive numbers, avoidance of 'blue' after electroshocks results in negative numbers.

For a series of these measurements the average of Δ and the standard deviation (not the standard deviation of the mean) was computed (Table 2). A *t*-test for significance of the differences ($\Delta \neq 0$) was performed. Although not quite appropriate, because it neglects the explicit performance of difference measurements, a χ^2-test of the total number of flies within a series of experiments is listed as well.

Table 1 lists the results from four different sets of experiments. In the first set, the flies were exposed in the training alley to first the yellow light then the blue light. Electroshocks were applied at either the first or the second funnel. In experiment II the order of lights in the training alley, was reversed. In experiment III there were no lights in the training alley, but lights in the T-unit, and experiment IV was run in complete darkness. This last experiment was meant to demonstrate that, with the exception of the light stimuli, the apparatus is completely symmetrical; no right–left bias was observed.

Table 1 lists the number of animals counted in a single experiment and its corresponding control without electroshock. The data are representative of the experiments performed. (Their statistical significance is evaluated through Table 2.) The data suggest that positive learning, that is, association of the optical stimulus with electroshocks and subsequent avoidance of a similar optical environment occurs. The results of these experiments cannot be explained by sensitisation by electroshocks. Because of the symmetrical experimental design, electroshocks both at 'blue' and at 'yellow', a pseudolearning control is unnecessary. That it is indeed the optical stimulus which is being learned is suggested by experiments III and IV which are not significantly different from their respective controls.

Table 2 shows the averages of a number of experiments with wild type *Drosophila* (strain Berlin) and the mutant *ebony*. The data clearly demonstrate a positive learning behaviour both for avoidance of the yellow light stimuli and for avoidance of the blue light stimuli. Although the effects are not large, the deviation from zero of the difference between experiment and control is highly significant in all cases. A sign test yields a high degree of significance. Out of 46 experiments only two cases of 'masochism' were found, while 44 cases indicated avoidance of the optical stimulus associated with the electroshock. In contrast, if the electroshock was not associated with a light stimulus (no lights in the training alley) no significant differences between experiment and control were found (Table 2).

The greater incidence of positive learning for the wild type in blue light probably results from a favourable relation between light intensity and physiological sensitivity in these circumstances. Further studies are necessary before more quantitative relationships can be established. It is to be expected though, that larger effects will be obtained if the flies are exposed to more than one training situation either at short intervals or on consecutive days.

It should be pointed out that the two light stimuli used in these experiments differ not only in colour but also in physiological rating. Thus, the experiments are not necessarily interpreted as proof of discrimination of the two colours[11]. There is independent evidence for colour vision of *Drosophila* obtained by Schümperli[13] as well as in this laboratory. However the data presented here are considered proof of associative learning in *Drosophila* with respect to optical stimuli.

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1 Benzer, S., *Proc. natn. Acad. Sci. USA*, **58**, 1112 (1967).
2 Benzer, S., *J. Am. med. Ass.*, **218**, 1015 (1971).
3 Götz, K. G., *Kybernetik*, **2**, 77 (1964).
4 Heisenberg, M., *J., comp. Physiol.*, **80**. 119 (1972).
5 Hotta, Y., and Benzer, S., *Nature*, **222**, 354 (1969).
6 Heisenberg, M., *J. exp. Biol.*, **55**, 85 (1971).
7 Campos-Ortega, J. A., *Z. Zellforsch. mikrosk. Anat.* (in the press).
8 Murphey, R. M., *Anim. Behav.*, **15**, 153 (1967).
9 Murphey, R. M., *Anim. Behav.*, **17**, 43 (1969).
10 Yeatman, F. R., and Hirsch, J., *Anim. Behav.*, **19**, 454 (1971).
11 Quinn, W. G., Harris, W. A., and Benzer, S., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
12 Hadler, N. M., *Biol. Bull mar. biol. Lab., Woods Hole*, **126**, 264 (1964).
13 Schümperli, R., *J. comp. Physiol.*, **86**, 77 (1973).

Identification of chemicals of snail origin that attract *Schistosoma mansoni* miracidia

MEASURES to control schistosomiasis (bilharziasis) aimed at either the molluscan vector or the trematode's larval stages might be enhanced by a better understanding of the intricacies of how miracidia and cercariae find and penetrate hosts. Previous work from our laboratory identified chemicals from mammalian skin that initiated penetration responses by *Schistosoma mansoni* cercariae[1]. Other earlier studies implicated amino acids and additional compounds as attractants of *S. mansoni* miracidia to the molluscan host, but did not show that these substances were derived from the snail[2]. Subsequently, based on Neuhaus'[3] and C. A. Wright's[4] indications that snails release substances into water that stimulate miracidia, several investigators[5–8] have confirmed that snails 'condition' water making it stimulatory or attractive to miracidia. Wright and Ronald[9] recently demonstrated that water conditioned by the snail *Lymnaea palustris* attracted miracidia of the rodent blood fluke, *Schistosomatium douthitti*, and that this snail-conditioned water (SCW) contained amino acids. Here we show that *Biomphalaria glabrata*, the molluscan vector of *S. mansoni*, the important parasite of humans, contributes amino acids

TABLE 1 Amino acids identified in snail-conditioned water.

Amino acid	(μmol l^{-1})
Aspartic acid	0.075
Threonine	0.075
Serine	0.382
Asparagine/glutamine	trace
Proline	0.052
Glutamic acid	0.046
Citrulline	0.028
Glycine	0.124
Alanine	0.153
Valine	0.055
Methionine	0.008
Isoleucine	0.033
Leucine	0.046
Tyrosine	0.025
Phenylalanine	0.027
Ornithine	0.015
Lysine	0.019
Histidine	0.014
	1.177
Tentative:	
Taurine = 0.054 μmol l^{-1};	
Urea = 2.51 μmol l^{-1}.	

Data represent means of two assays, each on a separate preparation of SCW. Amino acid concentrations were determined from standards prepared in the residue from 500 ml of spring water.

to the aqueous environment, and that these, and possibly other substances elicit chemotactic and chemokinetic responses by miracidia of this parasite.

The schistosome and hosts were maintained as described[1,2]. SCW was prepared by placing 100 snails (10 mm diameter minimum) in 500 ml of commercial spring water (Magnetic Springs Water Co., Los Angeles, California) for 2–3 h to minimise faecal output during the conditioning process, then rinsing with three 10 min changes of spring water to remove adherent materials. These snails were subsequently used to condition 500 ml of spring water for 18 h. No antibiotic was added to the spring water during conditioning to preclude the induction of amino acid release by the antibiotic. The snails were removed, the SCW filtered (0.45 μm pores), evaporated to 18.5 ml, acidified, and refiltered. The amino acid fraction was eluted from Dowex 50, evaporated to dryness, and taken up in 1.5 ml of 0.2 N sodium citrate buffer (*p*H 2.2) for analysis. Nineteen common amino acids were identified with an automated analyser (Table 1). No amino acid was detected in controls of 500 ml spring water (no snails) treated as above (three replicates). Two additional control samples were assayed similarly, except that a standard containing a known amount of each amino acid was added to the evaporated spring water. Neither amplification nor increase in numbers of the known peaks was detected. These results clearly demonstrated that the snails contributed amino acids to the water.

The stimulatory or attractive qualities of SCW before and after evaporation was tested by the method of Chernin[7] and always gave an immediate, strongly positive response. As this bioassay neither yields quantitative data nor necessarily shows a chemotactic response, we devised a choice-system bioassay consisting of a rectangular chamber (37 × 3 × 2 cm deep) mounted on the stages of two stereoscopic microscopes. The chamber contained 25 ml spring water. Miracidia (1–4 h old) were added in 1 ml of spring water to the centre of the chamber. For 20 min, two observers simultaneously recorded the number of miracidia entering the 3 cm field of view from the mid-chamber region on each end of the chamber; entries from the distal ends of the chamber were disregarded. This experimental design minimised the number of miracidia in these data that might be 'trapped' by scoring only those that entered from the mid-chamber region. The diffusing chemical may have established an active zone beyond the field of view and in this way caused miracidia leaving the mid-chamber region of the field of view to return to the source of the stimulus. Such a 'turnback' response indicates chemotaxis[2,7].

The time period began when 75 μl of substances to be tested were gently pipetted onto the surface at the centre of each field of view. Experiments were conducted in a darkened room at 22° C. Uniform, diffuse light was supplied by a 10 W neon tube centred 23 cm above the chamber's long axis. Preliminary experiments designed to standardise the system showed that this method gave a dynamic, quantitative analysis through direct observation of miracidial behaviour in the presence of both experimental and control substances.

Using this system, we demonstrated that the presence of SCW produced significantly more entries into the field of view than did spring water controls (Table 2). In addition, behavioural changes described by MacInnis[2] and by Chernin[7] were exhibited by nearly all miracidia entering the field containing SCW, but rarely in the control. In other experiments concentrated SCW was prepared in agar blocks[2], then bioassayed in the chamber against agar made with spring water. At the end of five 10-min experiments with these agar blocks, the ratio of miracidia in the fields of view was 10:1 (experimental:control). These results clearly demonstrated that the presence of SCW not only attracted miracidia, but also kept more miracidia in the vicinity of the source of the stimulus.

To ascertain whether or not the amino acids identified in SCW could elicit responses by miracidia, a mixture of amino acids was prepared at concentrations equivalent to that determined to be present in SCW (Table 1, taurine and urea were omitted). This mixture was also assayed with the amino acid analyser, and the concentration of each amino acid shown to be essentially as noted in Table 1. Bioassay of this mixture (Table 2) demonstrated that the amounts of amino acids produced by snails increased the number of entries into the field of view. This mixture appeared slightly, but not significantly, more effective than the SCW assayed (Table 2, $P > 0.1$). This difference might be explained by the fact that the size of snails used to prepare SCW in these trials was somewhat smaller than those used to prepare the SCW for chemical analysis. Such an interpretation is consistent with Chernin's results with SCW from snails of different sizes[7].

Table 2 also shows the results of double control experiments in which 75 μl of spring water were added to each end of the chamber. Comparison of these results with those of the controls run with SCW or the amino acid mixture demonstrated that the presence of the SCW or amino acids not only increased entries into the field of view, but also reduced the number of entries in the field opposite the chemicals. Such results indicate that amino acids and other chemicals released by snails cause miracidia to localise preferentially in

TABLE 2 Bioassay of 75 μl of snail-conditioned water and of an amino acid mixture equivalent to the amino acids determined to be present in it (Table 1).

Time (min)	Entries into field of view (mean ± s.e.)		No.	P
	Control	SCW		
10	22 ± 2	38 ± 6	(5)	<0.05
20	55 ± 4	78 ± 5	(4)	<0.05
	Control	Amino acid mixture		
10	21 ± 2	34 ± 4	(6)	<0.02
20	54 ± 2	89 ± 4	(6)	<0.001
	Control	Control		
10	23 ± 3	23 ± 3	(5)	>0.80
20	61 ± 6	65 ± 7	(5)	>0.60

No., Number of experiments (50 miracidia per experiment in 25 ml spring water); control is 75 μl of spring water; in the double control experiments 75 μl of spring water were added to each end of the chamber. Replicates were accomplished by alternating experimental and control additives in each end of the chamber. Probability, P, calculated from Students t test.

the region of the stimulant, and thus enhance the chances of a miracidium encountering a snail.

SCW contains many components other than amino acids. Preliminary experiments with a peptide fraction (obtained from SCW by the method of Nakano and Yamamato[10]) showed no attraction over a distance, but did elicit slight modifications of the normal miracidial swimming pattern. Preliminary analyses of SCW (transesterified with methyl alcohol) by gas-liquid chromatography indicated the presence of at least six peaks whose identity and ability to stimulate miracidia have not yet been resolved. Our results with the amino acid mixture, and those of Shiff and Kriel[6], who passed their SCW through a 'mixed-bed resin', suggest, but do not preclude the possibility, that inorganic ions need not contribute to attraction of miracidia.

Although amino acids or other substances may leach out of the mucus and faeces of the snails, amino acids also may be released from the epithelial cells of snails in an osmoregulatory function as discussed by Potts[11]. It may be noted that Chernin[7] reported that faeces of *B. glabrata* were not attractive to *S. mansoni* miracidia in the bioassay. C. A. Wright[4] found that faeces did stimulate miracidia, but such results must be interpreted with caution as any material removed from SCW may absorb sufficient amounts of the stimulative substances to elicit a response. Our results clearly proved, however, that the amino acids produced by snail hosts can serve as attractants for miracidia. How can such results be used to improve methods of controlling bilharziasis and other pathogens?

It is possible that amino acids (and other, as yet unidentified components of SCW) produced by snails, or released from plant materials during feeding by snails, keep miracidia, cercariae and snails within a limited focus helpful to completion of their life cycles. Attraction of snails to amino acids was proposed by MacInnis, but has not yet been tested[2].

Incorporation of attractants such as amino acids into larvacides and molluscicides, possibly into easily dispersed materials such as proposed by N. F. Cardarelli (United States Patent No. 3,417,181, December 17, 1968) may be used to attract both parasite and vector into a zone of effective pharmacopoeia. Alternatively, they might be attracted to particles containing insoluble poisons (which would be less dangerous to the ecological aspects of the environment) and induced in the case of the mollusc to feed upon such materials, or, in the case of the larval trematodes, to waste their energies and secretions in futile attemps to penetrate particles emanating attractants which also initiate penetration responses[2,7]. Amino acids and other substances released from processed plant material used as a schistosomacide or molluscicide, might also contribute to the success of such material when used in nature[12,13]. These possibilities, in view of the fact that the widely used molluscicide sodium pentachlorophenate is a repellant to snails[14], further provide support and impetus for future investigations on artificial or natural larvacides and molluscicides in combination with attractants.

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1 MacInnis, A. J., *Nature*, **224**, 1221 (1969).
2 MacInnis, A. J., *J. Parasit.*, **51**, 731 (1965).
3 Neuhaus, W., *Z. Parasitkde.*, **15**, 476 (1953).
4 Wright, C. A., *Proc. first int. Cong. Parasit., 1964*, **2**, 1058 (1966).
5 Kinoti, G., *Ann. trop. Med. Parasit.*, **62**, 382 (1968).
6 Shiff, C. J., and Kriel, R. L., *J. Parasit.*, **56**, 281 (1970).
7 Chernin, E., *J. Parasit.*, **56**, 287 (1970).
8 Wilson, R. A., and Denison, J., *Comp. Biochem. Physiol.*, **32**, 511 (1970).
9 Wright, D. G. S., and Ronald, K., *Can. J. Zool.*, **50**, 855 (1972).
10 Nakano, E., and Yamamato, S., *Dev Biol.*, **28**, 528 (1972).
11 Potts, W. T. W., *Biol. Rev.* (Cambridge), **42**, 1 (1967).
12 Lemma, A., Brody, G., Newell, G. W., Parkhurst, R. M., and Skinner, W. A., *J. Parasit.* **58**, 104 (1972).
13 Pellegrino, J., *Expl. Parasit.*, **21**, 112 (1967).
14 *Bull. Wld Hlth Org.*, **33**, (1965).

Stereopsis by binocular delay

HERE I report results which reveal a new form of binocular stereopsis sensitive to delays between the arrival of common input to both eyes instead of retinal disparity.

Until the work of Julesz[1] monocular form detection was commonly believed to be a prerequisite for the detection of binocular retinal disparity and so for stereopsis. Julesz showed monocular form detection not to be necessary by demonstrating cyclopean perception of targets in depth with random dot stereograms. Neither member of the stereogram pair conveyed any monocular information about the target. A continuous analogue of Julesz's random stereogram method is used in the present observations to show that neither monocular form nor binocular disparity is necessary for stereopsis.

Figure 1 shows that when both eyes are fixated on a target, each sees different areas of the foreground and background, a fact established by Hering's[2] demonstration of the Law of Identical Visual Directions. It follows that as the eyes rove at a constant fixation distance, they encounter the same background texture at different times, the delay being a function of the eyes' rate of traverse, the distance of the target and the distance of the background.

When they move from left to right, the left eye encounters parts of the background before the right. In the foreground, the situation is reversed. The right eye leads and the left trails. Both eyes encounter whatever is at the fixation distance at exactly the same time.

The apparatus presented each eye with some common (target) input at the same time, and some common (background) input at different times. Each eye viewed a point-plotting oscilloscope (Tektronix 602, P15 phosphor) controlled by a PDP8-E computer via specially designed interfaces. (For details see Ross and Hogben[3].) Polarised filters shield each eye from the other's view. The first target was an outline square (2 cm × 2 cm), presented to each eye simultaneously in repeated 10 ms bursts, at a rate above critical flicker fusion. The background was a continuous (or dynamic[4]) noise stream of single dots, distributed uniformly at random over the whole of an 8 cm × 8 cm display area, within which the target was centred. Dots were plotted at a rate of about 9 ms^{-1} whenever the target was not being plotted, so that each eye seemed to see a field of many dots, like a snowstorm, the distribution continuously changing. Each dot in the stream was shown to both eyes at the same rate, but at different times. One stream, so to speak, lagged behind the other.

It should be noted that delay here does not produce an effective disparity, as it would if successive points fell in order along a horizontal line, because points fall randomly in two dimensions. Effective disparity due to delay has been proposed as an explanation for the Pulfrich phenomenon..

When the delay between the eyes is below about 50 ms, the binocular view reveals a target roughly in the same plane as the noise field. When it is above about 70 ms, the noise field recedes well behind the target. The effect is vivid, and unmistakeable, even for some observers with poor stereopsis for displays based on disparity. Between 50 and

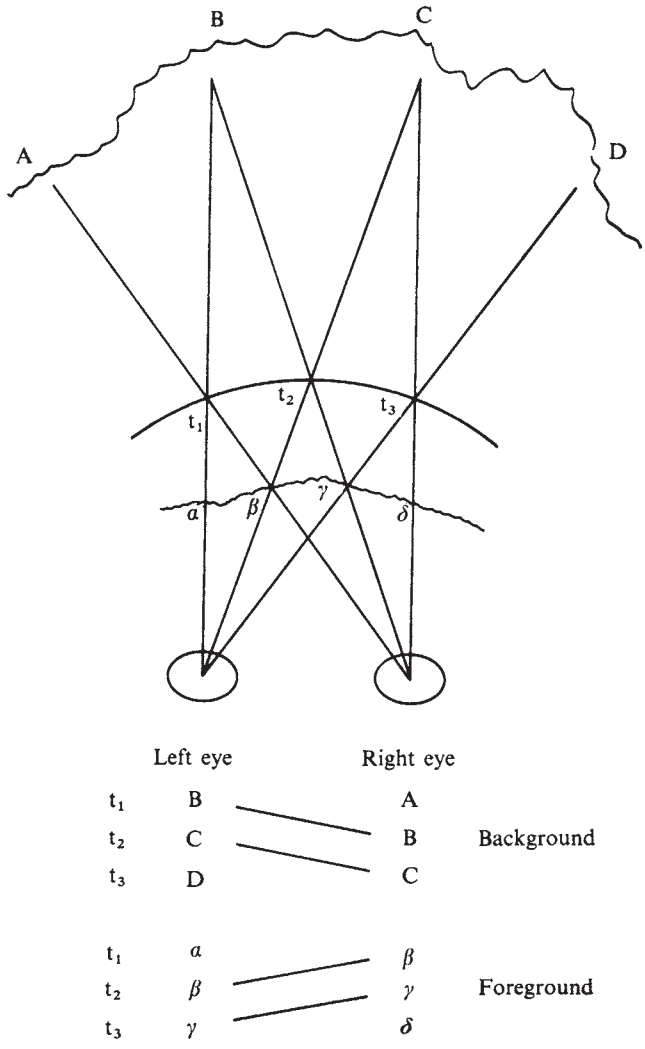


FIG. 1 View of each eye at different times. A, B, C and D are points in the background; α, β, γ and δ are points in the foreground. Because of motion parallax, rate of transverse over the background is more rapid than over the foreground.

70 ms, the situation is ambiguous, the background being seen alternately in the plane of the target, or behind.

Delay was pitted against disparity by shifting dots in the noise stream to give them a crossed disparity. Below 50 ms delay, disparity is dominant, the noise plane being seen in front of the target, and transparent. Above 70 ms, delay is dominant, the noise plane now lying behind the target and looking opaque. In the 50–70 ms range, the background alternates from in front to behind. The alternation does not seem amenable to voluntary control.

The monocular contour provided by the outline square can be eliminated by an analogue of the Julesz method, mentioned above. Points to be plotted to both eyes are generated at random within the 8 cm × 8 cm available display area. Points falling within a central square area (2 cm × 2 cm) are plotted, forthwith and simultaneously to both eyes. Points falling outside the central square area are plotted immediately to one eye, and buffered, to be displayed, at the same rate, but later, to the other eye. Each eye sees the same points at the same rate and distributed uniformly at random over the whole display area. All that differs is the time of presentation. There is no monocular distinction between target and background. The only mechanism which could determine that there was a distinction is one which had access to both fields of view, and which could register and recognise differences between them.

When delay in the background exceeds 70 ms, the target is seen standing in depth, in front of the background. The edges of the target are not so sharply delineated as when disparity is used to define a target but, as with disparity, there is no apparent motion of dots across the boundaries of the target. (See Julesz[5], and Ross and Hogben[3], for comparable effects with disparity). Of course, there is no similar confinement of motion within either monocular view. At delays below 50 ms, the target can be distinguished from the background, but not clearly and only faintly at differential depth.

When the background is composed of independent sets of points, so as to produce rivalry, both targets, the monocularly visible and the monocularly invisible, sit embedded in, rather than in front of a nebulous surround, thus eliminating the possibility that delay induces complete or partial rivalry and has its effect accordingly.

The observations reported here show that retinal disparity determines relative depth when input is received by the two eyes within a span of 50 ms, confirming the value determined by Ross and Hogben[3], for short-term memory in stereopsis. More importantly they show that delay alone can determine relative depth when the span for disparity is exceeded. It can do so even in the absence of monocular information about form, as can disparity.

The upper limit for delay has not yet been determined precisely, but indications are that it is near 2 s. At delays beyond 2 s the target is visible, but the background looks rivalrous, has the nebulosity characteristic of independent random point streams and is no longer a plane behind the target.

Many observational details fit the suggestion that the visual system handles common input with binocular delay as if it had been generated by eye movements (see Fig. 1). When the left eye receives input first, the background streams from right to left, as if the eyes were sweeping left to right. The target appears stationary. Direction of background movement is reversed when the right eye receives input before the left. At certain delays, there is also a lacy plane in front of the target, with movement always in a direction opposite to that on the back plane. As Fig. 1 shows, the eye leading in seeing background texture trails in seeing foreground texture.

Retinal disparity provides a fine sense of depth within a narrow spatial range, Panum's fusional area, and a narrow temporal span of about 50 ms (see Ross and Hogben[3]). We may speak of a fusional area and a fusional span, both limiting disparity in stereopsis. The results reported show that the visual system can also deduce relative depth, and direction of movement, from common input received at binocular delays beyond 50 ms. There thus seem to exist two binocular systems for depth, one operating within narrow spatial and temporal limits, to give fine resolution around fixation, and the other operating outside the temporal limits of the first to give a coarse indication of the structure of background and foreground space.

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1 Julesz, B., *Bell System Tech. J.*, **39**, 1125 (1960).
2 Hering, E., *Outlines of a Theory of the Light Sense*, Trans. from German original, of separate sections (Harvard University Press, 1964).
3 Ross, J., and Hogben, J. H., *Vision Res.* (in the press).
4 Uttal, W., *Percept. and Psychophys.*, **6**, 121 (1969).
5 Julesz, B., *Foundations of Cyclopean Perception* (University of Chicago Press, Chicago, 1971).

science on television

Visceral learning

John Wilson

Dr Neal Miller, of Rockefeller University, New York, believes that we can learn to control our blood pressure, and other regular bodily functions, just as we control the movement of our limbs—if only we try hard enough. With my overdraft in mind, I thought that my bank manager might benefit from the technique so when Yorkshire Television asked "What's Visceral Learning Anyway?" I settled back to learn the answer.

It was not long in coming. Before the opening credits had rolled there was Dr Miller's most successful hypertensive patient, a woman, telling all. She did not know how she did it, she said, it just happened (or rather more accurately, it later transpired that she provided explanations, such as thoughts of old Bogart movies, which her doctor discounted).

Having given the game away at the outset, the programme went on to relate the history of visceral learning, and did so very well. The phenomenon was introduced by way of examples (a young boy controlling the temperature of his hand, a gunshot victim regulating his blood pressure and Dr Miller himself wiggling one ear only), followed by a brief description of the relative importance of the somatic and autonomic nervous systems.

Dr Miller, we learned, first came to doubt classical theory on the independance of the autonomic system as a result of some very courageous experiments involving 16 human volunteers. When paralysed from the waist down by injections of curare or acetylcholine, these volunteers could still control their urine. If the bladder could be regulated in this way, he reasoned, might not other usually automatic functions of the body be controlled also?

For his experiments on rats, Dr Miller implanted electrodes into their brains so that he could reward them for a 'correct' response in blood pressure even when they were totally paralysed with curare. In living colour, the surgery involved must have turned off nearly as many sets as the recent party political broadcasts but to those of us inured to this kind of bloodshed it provided a fascinating insight into the procedure involved in this elegant experiment which, incidentally, took three years to develop. It was also good to see the ambiguities of the experimental work being admitted to the public and the importance of control experiments emphasised. In view of the *dénouement* to follow, this was just as well.

For the sombre truth about Dr Miller's experiments is that nobody has ever been able to verify them, although five years and 2,000 unfortunate rats later another researcher, Dr Barry Dworkin, is still trying. Various reasons for his failure were presented to the viewer and then demolished, just as they were gaining credibility.

Had the company supplying the rats been rearing its charges in too much luxury and thus making them less susceptible to stress? No, when the animals were subjected to what Dr Dworkin called the "summer camp" treatment by dropping then in ice-cold water (heaven preserve me from American summer camps) they continued to turn in contradictory results. Could the curare used in the original experiments have been altered? Apparently not—but perhaps a placebo effect was taking place.

This sort of criticism in a television science programme is rare and could have been handled in a most destructive manner but the "Discovery" team should be congratulated on its balanced view of a most interesting topic.

The programme did contain a rather surprising flaw, however. Against a picture of a row of oriental mystics, seated deep in meditation and with electrodes taped to their shining heads, we were told of their amazing ability to confound modern science, represented by the scribblings of an electroencephalogram. No sooner seen than gone, the achievements of these people surely deserved more than this cursory mention.

matters arising

African swells, magmatism and plate tectonics

Sir,—Gill[1] states that the 'basin-and-swell' topography of Africa is now widely regarded as the product of heat flow anomalies in the upper mantle. Are these anomalies fixed with respect to the Earth's rotational axis or not? My contention is that they are not. The great Mesozoic to late Tertiary magmatic provinces of eastern Africa become progressively younger northward, when the overall movement of the African plate was in general northward and north eastward; and the magmatic provinces of west Africa have remained in more or less the same place since mid Jurassic times.

My ice-floe analogy[4] was intended only to suggest that gentle lithospheric buckling might initiate the development of these broad thermal anomalies, or megaplumes, perhaps by enabling an asthenospheric up-current to develop, which would then be the source of magmatism and the main agent of crustal doming, and which could easily give rise to tensional features, such as rift valleys.

Gill quotes a figure of 10 km as the maximum possible wavelength for 'compressional warp structures', which seems improbable in a lithosphere 70–100 km thick. I also question whether the idea that gentle warping in response to stresses arising from interplate 'jostling' is invalidated by reference to post-glacial isostatic recovery rates.

It is therefore still not impossible that a small degree of pressure-relief partial melting could trigger development of asthenospheric upcurrents. Gill is correct in inferring that I emphasise the mantle as a source of much salic magmatism, but whether such magmas are produced directly by partial melting or indirectly by high pressure fractionation is not important—what is important is that the mantle is demonstrably the source of some phonolites and trachytes. I still prefer the partial

melting model, and Gill states that such a model requires low temperature salic melts to be sweated off from the mantle earlier than basaltic liquids. In east Africa, great effusions of phonolitic magma were among the ealiest manifestations of volcanism. In this connection, it is also worth noting that the difficulties of explaining the simultaneous availability of basic and salic magmas are just as great on a fractionation as on a partial melting model.

Yoder[2] has outlined a fractional melting mechanism to account for the contemporaneous occurrence of magmas of highly contrasted composition—in particular basalt and rhyolite, although there is no reason why similar arguments could not apply to alkaline basic and salic magmas. In a modified version of his earlier ideas, Bailey[3] has proposed that plate tectonic processes could give rise to uneven stress distribution, resulting in gentle warping of continental plates. If low melting constituents were drawn into the arches, the resulting lower density mantle would provide a density contrast for continued uplift as observed in major continental alkaline provinces.

Finally, whether or not these comments constitute a valid reply to the points raised by Dr Gill, the central thesis of my paper is unaffected: alignments of individual magmatic centres within a magmatic province can only very rarely be attributed to movement of the lithosphere over a stationary mantle hot spot.

Yours faithfully,

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1 Gill, R. C. O., *Nature,* **247,** 25 (1974).
2 Yoder, H. S., *Am. Miner.,* **58,** 153 (1973).
3 Bailey, D. K., *J. Earth Sci.,* **8,** 255 (1972).
4 Wright, J. B., *Nature,* **224,** 565 (1973).

Second Law of Thermodynamics

Sir,—In a recent article[1] Hillel proposes a statement of the Second Law of Thermodynamics which he claims is consistent with the time reversal symmetry of the microscopic laws of physics. Because the issues raised in this article nicely illustrate some of the fundamental misconceptions which continue to bedevil the subject of temporal asymmetry, I wish to make the following response.

In stating his form of the Second Law: "The entropy of a closed system tends to change monotonically with time", Hillel confuses the assumed underlying isotropy of time itself (absence of intrinsic preferred orientation) with the symmetry of physical processes with respect to time, a confusion which is in fact almost universal. The isotropy of time itself always enables us to replace the words "increasing entropy" by "decreasing entropy" in thermodynamic statements, for this merely amounts to an inversion of our conventions "later than; earlier than"—permitted provided these conventions are not referred to other physical processes (such as K^0 meson decay) which are asymmetric in time independently of thermodynamics. Hillel's remarks are thus a statement about time and not about thermodynamics.

Indeed, his is not a statement of the Second Law consistent with the symmetry of microscopic processes with respect to time, as alleged. For it is precisely the stated monotonic change of entropy in time which is the asymmetry of thermodynamics and which is apparently in conflict with the reversibility of atomic motion (Loschmidt's paradox). The fact that we are free to call the change an increase or decrease cannot alter the fact that the change is still asymmetric in time. Such a situation is well known to be incorrect, because it contradicts Poincaré's theorem, according to which all states in a closed system will be closely revisited eventually. Most laboratory systems soon reach equilibrium, after which fluctuations occur, during which the entropy both increases and decreases. These fluctuations often lead to observable effects such as Brownian motion.

A truly time symmetric statement of the Second Law, which is certainly necessary, has always been available:

If a closed system is in a random state of low entropy it is overwhelmingly likely to have been in a higher entropy state just before that moment and to be in a state of higher entropy just after that moment.

This statement resolves Loschmidt's paradox and is consistent with Poincaré's theorem. It also becomes consistent with our observations when it is appreciated that real systems are not permanently closed, but are branch systems from the main environment formed at a finite time in the past. Such systems are formed in low entropy states, and simply do not exist prior to their formation. Consequently, the monotonic entropy change referred to by Hillel is actually a consequence of the asymmetric formation of branch systems, which itself is related to the condition under which the universe emerged from the "big bang". It is actually possible to understand this condition in detail in terms of reaction thresholds of elementary particle processes on the one hand, and the peculiarities of gravitational thermodynamics on the other. I refer the reader to my forthcoming book for a full discussion of these cosmological topics[2].

Yours faithfully,

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1 Hillel, A. J., *Nature,* **242,** 456 (1973).
2 Davies, P. C. W., *The Physics of Time Asymmetry* (S.U.P., in the press).

Dr Hillel replies: On the basis of classical dynamics, one can show that a system in a random state of low entropy is overwhelmingly likely to be in a higher entropy state just before that moment and also just after. This is put forward by Davies as a statement of the Second Law which has always been available in the literature. It is, however, a theoretical statement which is still commonly thought to be at odds with the observations which we summarise in the Second Law. This is precisely the basis of Loschmidt's paradox. The theory allows the possibility of a system "prepared" at a certain time, whose entropy was decreasing monotonically at earlier times. This seems to be inconsistent with observations and is forbidden by the usual statement of the Second Law. At this stage it is argued that in practice the system cannot exist before its preparation. I do not find this argument helpful. It does not remove the asymmetry but merely restates it. Is it possible, in principle, to observe such a monotonic decrease in entropy, and if so would the observations be physically acceptable to us? I argue that the answer is yes and that our statement of the second law should be modified accordingly. The statement I propose is consistent with the reversibility of the laws of mechanics (as embodied by Davies's theoretical statement). Poincaré's theorem is not relevant when considering statistical tendencies during times much shorter than the Poincaré time.

I do not accept Davies's distinction between the isotropy of time itself (absence of intrinsic preferred orientation) and the symmetry of physical processes with respect to time. A physical law is symmetrical under time reversal if, for any allowed sequence of events, the time reversed sequence is also allowed by the law. My proposed statement was designed to satisfy this criterion, because thermodynamic observations are symmetrical in this sense, and that is why a thermodynamic time reversal produces no observable effect other than an apparent inversion of our verbal conventions "later than; earlier than".

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University of Manchester

obituary

G. Scatchard

GEORGE SCATCHARD was born in Oneonta, New York, on March 19, 1892, and died in Cambridge, Massachusetts, where he spent most of his life, on December 13, 1973. He received his AB degree from Amherst in 1913 and his PhD from Columbia in 1917. His PhD degree was in organic chemistry, on the synthesis and properties of quinazolines, but his interest shifted early to physical chemistry, and it is as a physical chemist, or, more precisely, as a thermodynamicist, that he established his reputation. He was Professor of Physical Chemistry at MIT for most of his career.

George Scatchard loved thermodynamics, its pure simplicity of expression, its certainty, its complete freedom from questionable assumptions. The vaguely understood effects of intermolecular interactions are always rigorously included in his equations as activity coefficients and their derivatives. Much of his theoretical work was concerned with the extraction of these non-ideal parameters from experimental data, with their interpretation, and with extrapolation methods that would eliminate them and permit exact determination of the simpler parameters, such as standard electrode potential and molecular weight, that enter into the definition of ideal thermodynamic behaviour. In the laboratory, George Scatchard dedicated himself to valiant efforts to match experimental precision to the precision of the equations he used for analysis. A characteristic of all his work, painfully discovered by many less patient experimenters who have sought to use his methods for analysis of their own data, is his ingenuity in devising plotting procedures that amplify experimental error and reveal at a glance the uncertainty of an extrapolation or in the drawing of a tangent.

George Scatchard was a severe critic. He did not necessarily expect others to match his own precision of thought or experiment, but he expected them to try their best. When he detected gross confusion or misconceptions, and especially when they masqueraded behind a facade of glib showmanship, he could be merciless. He will not be forgotten by anyone who presented seminars at the Harvard-MIT Physical Chemistry Colloquium when he was present. There was a perpetual frown on his face, deepening in intensity at each point where the speaker was glossing over theoretical or experimental difficulties. At the end of the seminar there was relief when he asked an innocuous question, and (depending on the personality of the speaker) either anger or a silent resolve never to err again when he rose to expose a fallacy. There are undoubtedly some who remember George Scatchard with less than affection as the result of such an encounter, but there are many more who are grateful for his example and for the effect it had of raising their own standards of what is and what is not a valid piece of scientific research. As might be expected, George Scatchard's scientific papers often had the purpose of defining parameters and establishing methodology.

From 1930–40 he was mostly concerned with the thermodynamics of solutions, and especially of aqueous solutions of electrolytes beyond the range of applicability of limiting laws. During this period he developed the isopiestic method for evaluating osmotic and activity coefficient, and wrote definitive papers on the use of electromotive force and freezing point depression. He also wrote purely theoretical papers including a pioneering paper with John G. Kirkwood, during tenure of a Guggenheim Fellowship in Leipzig, on the electrostatic interactions of zwitterions.

During the Second World War, he divided his time between the Manhatten project of the AEC and the blood plasma fractionation project at Harvard directed by E. J. Cohn, who had been a fellow undergraduate at Amherst and was a life-long friend. The latter work led to a general interest in proteins and to his classic papers in 1946 on the state of equilibrium across a semipermeable membrane. These papers were written to provide a sound foundation for osmotic pressure measurements of protein solutions at finite concentrations, but of course apply quite generally to all thermodynamic properties of multicomponent macromolecular solutions. Explicit extension to sedimentation equilibrium was made by Scatchard himself in 1954. The same basic theory provided the foundation for Scatchard's experimental work on the binding of ionic ligands to proteins and their interpretation in terms of specific binding sites and association constants, as well as for several papers on transport across semipermeable membranes.

There are very few scientists whose work one can legitimately compare with that of the handful of all-time great intellects. Such a comparison can be made, without appearing presumptious, between George Scatchard and J. Willard Gibbs. Nor is it derogatory to George Scatchard, in making this comparison, so say that Gibbs led the way and Scatchard followed for Scatchard himself said so. Few have the mental discipline that is required to go beyond a comprehension of what Gibbs accomplished, to an understanding of how he did it, to a sufficient insight into his thought process to be able to emulate it and to apply it to new and complex situations and George Scatchard was unique in his generation in his ability to do so. Without him many of the fruits of Gibbs' work would not have been gathered so soon.

C A Coulson

JUST over a century ago, T. H. Huxley wrote a paper in which he described the liberally educated man as one "who has been so trained in his youth that his body is the ready servant of his will and does with ease and pleasure all the work that, as a mechanism, it is capable of: where intellect is a clear cold logic engine with all its parts of equal strength and in smooth working order . . . whose mind is stored with the great and fundamental truths of Nature . . . and who, no stunted ascetic is full of life and fire, but whose passions are trained to come to heel by a vigorous will, the servant of a tender conscience; who has learned to love all beauty, whether of Nature or of art, to hate all vileness and to respect others as himself". It is ironic that an atheist should have so adequately encompassed the life and style of Charles Coulson that, provided 'Christian' is substituted for 'tender' no better words could be found for his epitaph. This remarkable man was born in 1910 and died in Oxford on January 6, 1974, steadfast to the end in his adherence to his principles and beliefs and in his sense of duty to others so that his last concerns were not for himself but for those at home and in his department who were, in diverse ways, dependent on him.

He entered Trinity College, Cambridge, from his local Grammar School as an open scholar and duly and predictably collected his first in the Tripos examinations. His first paper in 1931 was essentially an attempted criticism of one of the redoubtable Harold Jeffreys' conclusions and its writing epi-

tomised his unfettered pursuit of truth even if it led him into conflict with higher authority. In due course he was elected a Research Fellow of Trinity sharing this distinction with two physicists also with Methodist connections, the late L. H. Gray FRS and D. E. Lea. Their scientific interests converged in the then embryonic study of the action of ionising radiation on living systems. Lea and Gray went on to make most distinguished contributions to this field, but Coulson's was not particularly outstanding and he turned to the newly emerged subject of theoretical chemistry, then broadly defined as the application of Wave Mechanics to molecular structure and bonding, which occupied him for the last 37 years of his life successively as lecturer in University College Dundee, ICI Fellow in the Physical Chemistry Laboratory at Oxford, Professor of Theoretical Physics at King's College London, Rouse Ball Professor of Mathematics at Oxford (for about 20 years) and finally as the foundation Professor of Theoretical Chemistry at Oxford.

A combination of enthusiasm, great industry, fluency and an uncanny judgement of when to approximate made him a most prolific author (three books and over 300 papers). He touched on many chemical problems such as aromaticity, conjugation and hyperconjugation, charge distribution, shapes of molecules in ground and excited states. If his role initially was often to provide *post hoc* quantum-chemical justifications for many qualitative and semi-quantitative relations empirically perceived by chemists, he correctly foresaw that, once the computational obstacles had been overcome, the latent predictive powers of quantum chemistry would be realised and that it might outstrip instruments in quantitative precision.

Coulson will perhaps be remembered more as an unusually gifted teacher than a great researcher. He liked young people and they liked him—in their patois he 'related'. He had great sympathy for the intellectual difficulties of beginners and spared no pains in helping them. He loved lecturing and was a brilliant expositor, leaving his audience eager for more and confident that he could help them to an understanding which would enable them to select and solve problems. His summer schools in theoretical chemistry were world famous and a powerful influence in the dissemination of ideas and the development of the subject. They augmented the already strong attraction of Coulson's department for overseas visitors who often seemed to predominate in his research group.

Obituaries in *Nature* emphasise scientific achievement but it would be unjust to Coulson's memory not to recall that he was a deeply concerned Christian. This was manifested not only in his lay preaching to small congregations in insignificant Methodist Chapels or his public lectures to sophisticated audiences as in his Gifford lectures, but also in his willingness to play very active parts in organisations—such as a Vice-President of the Methodist Conference or as a working Chairman of Oxfam.

Honours were poured on Coulson but are not enumerated here for he would have preferred to be remembered simply as a practical idealist anxious to add to the sum of human happiness and understanding by advancing knowledge and by teaching and helping others, all of which he achieved abundantly.

Announcements

Appointments

J. Bardeen, H. B. G. Casimir, L. E. F. Neel, Sir Rudolf Peierls and Sir Alan Wilson have been elected Honorary Fellows of the **Institute of Physics.**

Sir Michael Perrin has been appointed a Trustee of the **British Museum** (Natural History).

Awards

The Council of the Institute of Physics has awarded the **Guthrie Medal and Prize** to **R. L. Mossbäuer** of the Institut Max von Laue-Paul Langevin; the **Rutherford Medal and Prize** to **A. E. Litherland** of the University of Toronto; the **Glazebrook Medal and Prize** to **B. J. Mason** of the Meteorological Office; the **Charles Vernon Boys Prize** to **P. G. H. Sanders** of the University of Oxford; the **Maxwell Medal and Prize** to **S. F. Edwards** of the University of Cambridge and the **Max Born Medal and Prize** to **W. Greiner** of the University of Frankfurt.

The Councils of the French Physical Society and the **Institute of Physics** have awarded the **1974 Holweck Medals and Prizes** to **A. Hewish** of the University of Cambridge and **P. Nozieres** of the Institut Max von Laue-Paul Langevin.

The **Royal Society** has awarded the first **Herschel Medal** to **P. Wild** of the CSIRO Division of Radiophysics.

Erratum

In Fig. 1 of the article "Nitrosocarbaryl as a potent mutagen of environmenal significance" by R. Elespuru W. Lijinsky and Jane K. Setlow (*Nature*, **247**, 386; 1974), the vertical axis should have been labelled mutants/survivor. On line 17 of the figure legend 0.01M NC should read 0.01mM NC.

Corrigendum

In the article, "Mechanism of denaturation of haemoglobin by alkali" by M. F. Perutz (*Nature*, **247**, 351; 1974) on page 343, paragraph 3, lines 9 and 10 of the section 'Probable mechanism' should read "Alkali denaturation of human oxyhaemoglobin has a negative entropy of activation . . ." and the first author of ref. 29 was misspelt and should be Air, G. M.

International meetings

April 8–10, **18th Annual Meeting of the Ecological Genetics Group** (G. E. Marks, The John Innes Institute, Norwich, UK)

April 8–10, **Course on Quantitative Microscopy for Materials Scientists** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford OX1 3HA)

April 8–10, **8th Thin Films Conference** (The Meetings Officer, The Institute of Physics, 47, Belgrave Square, London SW1X 80X)

April 8–11, **The Engineering Uses of Coherent Optics** (Mr E. R. Robertson, Department of Mechanics of Materials, University of Strathclyde, Glasgow)

April 8–12, **International Symposium on Wound Healing** (Secretariat International Symposium on Wound Healing, c/o Holland Organising Centre, 16, Lange Voorhout, The Hague, The Netherlands)

April 9, **Society for Drug Research Symposium on Essential Hypertension** (Dr A. B. Simmonds, Chelsea College, Manresa Road, London SW3)

April 9–10, **Microstructure and Physical Properties of Non-metallic Materials** (Dr D. Dew-Hughes, Department of Physics, University of Lancaster, Lancaster)

April 9–11, **Gels and the Gelling Process** (The Faraday Division, The Chemical Society, Burlington House, London W1V OBN)

April 10, **Applications of Remote Sensing—Data Processing and Analysis** (R. W. Laing, Electro-Optics Division, Hawker Siddley Dynamics Ltd., Manor Road, Hatfield, Hertfordshire AL10 9LL)

April 15–16, **11th Annual Rocky Mountain Bioengineering Symposium** (Clifford D. Ferris, The University of Wyoming, College of Engineering, Laramie, Wyoming 82070)